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AN EXPERIMENTAL HISTOLOGIC STUDY OF HYPERVITAMINOSIS D.*

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Soon after the discovery of the similarity of Vitamin D to irradiated ergosterol, several investigators became concerned with the action of excessive doses of irradiated ergosterol.[1-5] It was generally agreed that the compound exerted its effects by its influence upon calcium and phosphorus metabolism, presumably through some intermediary hormonal mechanism.[6,7] It was proved that the compound had no serious toxic action except when given in doses far in excess of those required for prevention or cure of rickets due to a deficiency of Vitamin D.[8] Finally, it was established that the principal pathologic changes attributable to excessive doses in man or animal were in the form of abnormal deposits of calcium in many organs and tissues.[9-11] Having settled practical matters concerning the clinical use of the compound, interest in hypervitaminosis D subsided, and except for the studies of Follis, little attention has been given to the subject since 1935.[12,13]

Our interest in hypervitaminosis D arose as a result of the emphasis of Wolbach, Bessey and Fell upon the use of avitaminoses and hypervitaminoses as tools for study of pathologic sequences and their relations to the locus or nature of action of vitamins.[14-18] Sequences in human disease with which we have been concerned relate to so-called degenerative changes which are especially conspicuous in connective tissues and more common in arteriosclerosis than in any other human disorder.[11,19] The lesions described in hypervitaminosis D seemed to offer a promising source of information in further study of these non-inflammatory degenerative sequences.[2-5,8] Hence, histologic studies of

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the distribution and evolution of lesions in rabbits given excessive doses of irradiated ergosterol were undertaken with special attention to changes in the vascular system.

Methods

The viosterol used in the experiments was a solution of irradiated ergosterol (15 mg. per ml.) in peanut oil. (Abbott Laboratories, Chicago, Ill.) The potency was assayed as about 10^6 U.S.P. units per ml. This was given intramuscularly in doses of 0.1 ml. (10^5 U.S.P. units) at daily, biweekly or triweekly intervals for periods as long as 6 to 8 weeks. The regime was varied at lower dosages so that minimal and maximal pathologic effects could be defined and their evolution recognized over a period of several months.

Male albino rabbits from a single stock, about 5 pounds in weight and 3 months old, were used. Records of weight and analyses for serum calcium, phosphorus and cholesterol were made at intervals of about 2 weeks. The animals were fed a Purina Rabbit Pellet diet with fresh vegetables. For control purposes, complete microscopic studies were made of 75 normal rabbits from the same stock, of the same age and maintained on the same diet.

Since the initial plan was to produce maximal pathologic effects, the dose was regulated at levels just below the quick lethal range. Hence, most animals lost weight and died within 6 weeks. Later, the plan was to analyze minimal, sublethal and early pathologic effects, and animals were either sacrificed at any early stage or given viosterol at longer intervals and sacrificed at a later stage. Complete necropsies were done and a record of the gross findings made. All tissues and organs were fixed in formaldehyde (4 per cent U.S.P.) and studied microscopically. A series of 54 animals were suitable for the purposes of this report, with 49 additional animals on other dietary and viosterol regimes available for supplementary and confirmatory observations.

Results

Gross Pathologic Changes

The pathologic processes attributable to hypervitaminosis D varied with the dosage of viosterol, the length of the period between doses, duration of the regime, and the occurrence of intercurrent infections.

Minimal conditions for production of significant generalized calcinosis were a duration of 8 days and a total of 300,000 units given in 3 equal doses at intervals of 2 days. These conditions were usually complicated by intercurrent chronic purulent pyelonephritis of a type occasionally encountered in control animals. Among animals free

from extraneous disease, except hepatic coccidiosis, the total minimal effective dose was 500,000 to 600,000 units. This dose produced generalized calcinosis of increasing severity as the time of administration in periodic equal doses was extended to at least 3 weeks. The severity of the disease was further increased as the biweekly administration of doses of 100,000 units was continued beyond 3 weeks. This regime generally led to anorexia, loss of weight and death within about 6 weeks. Accurate control of the rate of development of pathologic processes was also obtained, so that prolonged participation of tissue elements in mechanisms of resorption, repair and regeneration might be studied, but this required careful regulation of dosage over a period of about 6 months.

Significant gross anatomic changes were encountered only in animals with considerable disease, manifest by changes in bones or deposition of calcium in numerous extra-osseous tissues. These could not be correlated with any consistent changes in values for cholesterol, calcium, or phosphorus in the serum.

The changes in bones varied with the severity and duration of disease. In general, the bones were more brittle than normal (Fig. 9). The increased fragility was due largely to resorption of cortical bone, often sufficient to allow mechanical deformity of bodies of vertebrae so that partial collapse and abnormal cortical contours were occasionally conspicuous. In these cases, the trabecular structure was compact and the marrow spaces reduced so that the internal structure of the vertebral bodies resembled that found in human osteosclerosis and Paget's disease (Fig. 10).

The amount and distribution of abnormal deposits of calcium in extra-osseous tissues also varied with the duration and severity of the disease. In mildly affected animals there were no gross changes. With increasing severity of the disorder, calcium salts began to appear as white streaks which acquired an opaque brittle or granular character. The deposits conformed to anatomic planes and structural outlines of affected tissues. They were most conspicuous in the aorta and its major branches, being maximal proximally and diminishing distally in a consistent pattern.[2,20] The deposits were in the media, usually in the form of continuous sheets or as a series of discontinuous rings with aneurysmal deformities of the wall. Calcium deposits were less conspicuous in the kidney, where bandlike granular deposits, were noted, first in the inner cortex and later, as the calcified zone of the innermost cortex spread toward the capsule, as one or more convex parallel bands in the periphery of the medulla (Fig. 7).[4]

The next most common location of calcium deposits was in muscle (Fig. 2). The deposits coincided with white streaks which followed the pattern of orientation of muscle cells or distribution of arteries. They were less common in skeletal than cardiac muscle, but frequently were clearly distinguished in the diaphragm and smooth muscle of special parts of the alimentary tract. The esophagus was never affected. The walls of the ileum and colon were occasionally altered, but this never occurred unless there were extensive deposits in the muscular wall of the stomach subjacent to the acid-secreting mucosa (Fig. 5). The acid-secreting gastric mucosa also commonly contained deposits of calcium but less frequently than the subjacent musculature. Calcium deposits were not found in the mucosa or muscle of those parts of the stomach which have no acid-secreting function.

Another common location of calcium deposits was in the respiratory tract. The tracheal and bronchial cartilages were often rigid and calcified, and thin plaques of calcium were at times recognized in the mucosa of the larger respiratory passages. Calcium deposits were rarely found in the media of large pulmonary arteries but were fairly common in the walls of pulmonary veins. In connection with these changes, pulmonary emphysema and decreased elasticity of the alveolar structure were usually demonstrable.

Other tissues and organs occasionally contained gross traces of calcium salts, but in many locations the presence of the mineral deposits was established only by microscopic studies.

Microscopic Pathologic Changes

Heart

Deposition of calcium in the heart was usually associated with a peculiar inflammatory reaction similar to that described in rats (Fig. 1).[1,8] In general, the calcification potential of various cardiac structures in order of decreasing intensity and frequency was as follows: internal elastic membrane of coronary arteries, smooth muscle of coronary arteries, fibroelastic tissue of the endocardium, and cardiac muscle. Calcium deposition in cardiac muscle cells was confined initially to the "A" disks of the myofibrils. As the amount of calcium increased, the "I" disks were involved, and later the interfibrillar compartment was affected so that the muscle cell appeared to be impregnated uniformly with calcium. At times, as much as one third of the myocardium was involved, but as a rule the calcified muscle cells were distributed irregularly around the coronary arteries or in the subendocardial region (Fig. 2). This localization seemed to be determined partly by the

common occurrence of a low-grade inflammatory reaction (Fig. 1). At times, the reaction was principally in the form of arteritis and periarteritis, characterized by swelling of collagen, perivascular proliferation of histiocytes, and formation of focal nodular lesions which resembled Aschoff bodies. Usually these lesions lay either adjacent to or around cardiac muscle cells in various stages of degeneration, calcification, and resorption. Their incidence increased with the level of dosage of viosterol and perhaps with the intrusion of intercurrent infections, though data concerning the role of infections were too meager to justify final conclusions. Suffice it to say that control animals dying of the same infections had no lesions of the type described.

The presence of an active periarteritis and myocarditis may have had something to do with the distribution of calcified cardiac muscle cells, but there seemed to be little relation between the inflammatory reactions and calcification of the media of coronary arteries. The main coronary arteries were free from inflammation, and calcium deposits in the internal elastic membrane or smooth muscle of the media were more conspicuous in these vessels than in the smaller intramyocardial branches which exhibited inflammatory reactions. In some instances, the deposition of calcium was so great that it was detectable in coronary arterial branches of all dimensions. This process was never uniform in arteries of equal dimensions but involved certain arterial branches more severely than others.

Calcification of the fibrous or fibroelastic stroma of the heart was never impressive in valves or in the myocardium. The endocardium, especially of the left atrium, was occasionally severely calcified, the process being more pronounced in elastic networks than collagenous fibrils.

Respiratory Tract

The sequences and pattern of calcification in the respiratory tract were of great interest. The earliest deposition, other than that occurring in the tracheobronchial cartilages and goblet cells, was in the fibroelastic stroma of the tracheal mucosa. The calcium deposits were distributed at regular intervals just below the basal layer of epithelial cells. As a rule, the basement membrane and underlying fibroelastic tissues were affected. In more advanced cases, the deposits involved the entire basement membrane and variable amounts of subjacent fibroelastic tissue, as deep as the plane of the mucosal vascular plexus (Fig. 3). Only rarely did the calcium deposits extend in continuity from basement membrane to the perichondrium of cartilaginous rings. Invariably, however, in these severe lesions there was excessive calci-

fication of the cartilaginous matrix. When this occurred, the calcified zones of cartilage lay adjacent to those of the mucosa, and at times there was erosion and stromal penetration of the margins of calcified cartilage. This was not followed by osteogenesis. On the contrary, the stromal invasion stimulated resorption of mineralized matrix and regional proliferation of young cartilage cells.

Calcification of the subepithelial tracheobronchial stroma was often preceded or accompanied by swelling and distortion of collagenous fibrils, but customary signs of inflammation were usually absent. However, once calcification had occurred, reparative sequences were at times recognized. These seemed to be initiated by two factors. One was the fragmentation of calcified fibroelastic tissue and basement membranes, leading to local proliferation of histiocytes and encapsulation of displaced degenerated calcified tissue by histiocytes and multinucleated giant cells. As this occurred, the subepithelial basement membranes and adjacent mucosal stroma were usually slowly regenerated. When the reparative process was unduly active, the epithelium regressed to an undifferentiated type of columnar cells which regenerated as either a characteristic respiratory epithelium or a metaplastic epithelium of stratified squamous type. This form of epithelial behavior called to mind the sequence described by Wolbach in the course of deprivation and restoration of vitamin A.[16-18] The second factor initiating reparative reaction was the deterioration of epithelial cells which followed calcification of mucus in the secretory vacuoles of goblet cells (Fig. 3). These cells, in the absence of any signs of diminished viability, inflammation, and undue calcification of subepithelial basement membranes or deeper stroma, were occasionally transformed into spherical, concentrically laminated, calcified crystalline structures. This form of primary epithelial calcification usually stimulated a regenerative reaction in which proliferation of basal cells led to atypical orientation of the new epithelium in relation to rigid calcified basement membranes. Ordinarily this activated the regional stroma which produced an entirely new structure with characteristics of the original basement membrane. The new membrane was interposed between the discontinuous calcified original basement membrane and the proliferating epithelium. As this occurred, the displaced calcified membrane became encapsulated by histiocytes or incorporated in the cytoplasm of multinucleated giant cells.

Despite the apparent interference of these changes with tracheobronchial function, little associated inflammation was provoked. A low-grade chronic tracheobronchitis, characterized principally by an

infiltration of plasma cells in the respiratory mucosa, was found in a few animals. This followed the most severe instances of stromal and epithelial calcification. It was never encountered in control animals and was regarded as a specific feature of hypervitaminosis D.

Not all parts of the tracheobronchial tree were equally affected by the calcific changes. The process developed more readily in the trachea and, as the disease became more severe, spread distally. Hence, in mild cases the principal changes were in the trachea. In moderately severe cases, the lesions were conspicuous as far distally as the terminal bronchi supported by cartilage. In the most severe cases, the process extended all the way into the walls of the alveolar ducts and alveoli. At times, the calcification of the terminal structures was very advanced, with involvement of pulmonary elastic tissue and the walls of interalveolar capillaries and pulmonary venules (Fig. 4). The alterations led to diminished elasticity with increased rigidity, relaxation, and fragility of alveolar structures. Thus, early stages of emphysema became conspicuous, and although peripheral pulmonary changes were usually associated with advanced tracheobronchial calcification, the relationship was not always encountered and was by no means quantitative. It was clear that factors which determined the locus and quantity of tracheobronchial calcification did not necessarily operate equally in determining the distribution and magnitude of calcification in alveolar walls and pulmonary vascular channels.

Alimentary Tract

The upper alimentary tract was studied more carefully than the lower tract so that conclusions concerning changes distal to the duodenum were only tentative.

The esophagus showed no evidence of disease.

The stomach was a common site of severe calcification but only in special locations; namely, the part of the gastric wall concerned with support, nutrition, and the specific performance of acid secretion (Fig. 5). This part encircled the cardiac orifice and occupied the principal part of the fundus and greater curvature. The mucosa of the lesser curvature and the pyloric region contain few if any parietal cells which are generally held to be responsible for secretion of hydrochloric acid. The parts of the gastric wall which were calcified are therefore spatially and presumably functionally integrated. Calcium in the muscular wall was usually most conspicuous, and there was concurrent calcification of the mucosa and arterial system. Deposits of calcium in the mucosa

usually appeared first in the *tunica propria* and basement membranes adjacent to the common junction of branches of the compound glands in the midmucosal regions. Occasionally, cells lining the glands were calcified, and at times calcified concretions lay in the lumens of glands. Later, stromal calcification also occurred in other locations, especially near the muscularis mucosae. In connection with the stromal deposits, calcified walls of small arterioles were conspicuous, and at the level of the muscularis mucosae and submucosa, calcification of walls of larger arterioles and small arteries was still more prominent. Walls of larger arteries deeper in the *tunica muscularis* and serosa were less severely calcified although calcium deposits in the internal elastic membrane and subjacent media were common. In these vessels, fibroelastic intimal proliferation was apparent, always overlying calcified or fragmented stretches of abnormal internal elastic membranes or subjacent media.

Calcium deposition in the *tunica muscularis* and the muscularis mucosae of the stomach occurred in a characteristic sequence and pattern. Major deposits lay in the cytoplasm of smooth muscle cells which seemed, initially at least, to be structurally normal. The smooth muscle cells were ordinarily affected in groups, and with increased calcification only the bare outlines of initial structure remained. In its place there were rows of curious calcified bodies which resembled strings of beads without recognizable origin in cytoplasmic structure (Fig. 6). Minor deposits of calcium appeared in tortuous curved structures which at times resembled fibrous or elastic elements and at other times minute canaliculi or autonomic nerve axons. Further study will be required for the identification of these calcified filaments, fibers and other structures.

The pattern of transmural calcium deposition in elective sites in the stomach disappeared with the transition of gastric mucosa to the type which secretes no acid. Indeed, the entire thickness of the gastric wall just beyond the level of mucosal transition was normal in all cases. In other words, all arteries, nerves, smooth muscle, mucosal glands and stroma in the non-acid-secreting part of the stomach were free from calcium deposition.

Immediately beyond the pyloric sphincter, which at times contained a few clusters of calcified smooth muscle cells, a different pattern of calcification was encountered. Here, the duodenal mucosa, *tunica muscularis* and muscularis mucosae were ordinarily spared while calcium deposits appeared conspicuously in the walls of small arteries and arterioles. Distal to the duodenum the only important calcium

deposits were in the *tunica muscularis* of the ileum and especially the colon. The deposits varied greatly in amount and position. Mucosal and arteriolar calcification was indistinct or absent, except in the colon, in the reticulum of lymphoid follicles or in the basement membranes of interglandular surface epithelium. There was a peculiar concentration of calcium in and around ganglionic plexuses in the *tunica muscularis* of the colon. Other plexuses were uninvolved. Mucus-secreting goblet cells, commonly calcified in the respiratory mucosa, were unaffected throughout the ileum and colon.

Urinary Tract

The kidney was also a common site of severe disturbance and was affected in all cases in which there was significant calcification in other tissues.[3,4,8] The renal calcification was confined principally to aggregates of functionally related units. For instance, nephrons were not involved equally or in a random manner. Those first affected were nearest the medulla, and from this region the process spread to involve the more peripheral nephrons but seldom reached those in subcapsular areas (Fig. 7). In connection with this curious wave of calcification there was a similar spread in the medullary tubular areas corresponding to the distribution of loops of Henle.

The sequence of changes in nephrons was also of interest. The epithelium of the convoluted tubules seemed most susceptible; the cells were frequently solidly impregnated with calcium often disposed in a spherical, concentrically laminated pseudo-crystalline fashion, occupying and expanding the original volume of the cytoplasm and nucleus. Intraluminal calcified protein casts were also encountered in these tubules. The basement membranes of the convoluted tubules had a conspicuous affinity for calcium so that at times they were more heavily calcified than the epithelial cells. This affinity was prominent beyond the limits of the convoluted tubules, especially in the stroma of Bowman's capsule. Less often, the subendothelial membrane of glomerular arterioles was affected.

No conspicuous calcification of tubular epithelium and basement membranes of the kidney appeared without calcification of the related arterial system. The large extrarenal arteries were least involved, although calcification of internal elastic membranes and subjacent patches of medial structure was usual. Where the extrarenal arteries branched to enter the cortex, near the corticomedullary junction, pathologic changes became more prominent. The walls of arteries and arterioles supplying the nephrons undergoing mineralization were fre-

quently solidly impregnated with calcium so that mural structure was hardly recognizable (Fig. 15). The process of arterial calcification ordinarily extended in continuity to the glomerular tuft (Fig. 8). Involvement of the glomerular arteriolar plexuses was uncommon except when calcium was deposited in spherical masses scattered throughout the tuft in swollen glomerular endothelial cells. The postglomerular arterioles and venous channels were spared, except in the most severe instances of diffuse calcification. This was equally true of all arterial and venous systems connected with the nephrons of the peripheral half of the renal cortex.

The most remarkable feature of the renal lesions was their distribution. Of interest also, however, was the absence of expected signs of inflammation. Nor was there ever more than a trace of the reparative reaction one would anticipate in response to the observed magnitude and duration of renal damage. The disease was essentially indolent, progressive and degenerative.

The remainder of the urinary tract was not studied carefully, but ureters and renal pelves were often included in the sections. The ureters were always normal. The pelves ordinarily contained subepithelial deposits of calcium, occurring at intervals in the fibroelastic connective tissue and among bundles of smooth muscle cells. There was no inflammatory reaction preceding calcium deposition although the stromal fibers were often swollen and nodular. Following calcium deposition, there was in some instances a proliferation of histiocytes resembling the reaction encountered in similar calcified tissues in several other locations.

Vascular System

Particular attention was given to a study of the vascular system.[2,5] The earliest evidence of hypervitaminosis D was in the proximal aorta and changes were always found here, whenever there were calcific deposits in other locations such as the tracheobronchial mucosa, gastric wall and kidneys. As the disease became more severe and prolonged, calcium deposits appeared in the midaorta, then in the distal aorta, and at about the same time, in various aortic branches.

Microscopically, the initial calcium deposits in the aorta were in and along the innermost elastic membranes and delicate intermembranous fibrils or matrix (Fig. 11). With increasing duration and severity of the disease, calcification increased in depth and magnitude. In advanced cases the inner third of the media of the proximal aorta was calcified in continuity, and there were discontinuous annular zones of calcification in the middle third of the media. The depth of pene-

tration of calcification in continuity decreased slightly with increasing distance from the aortic valve but not in proportion to the decrease in thickness of the media. Hence, in advanced cases, the full thickness of the media of the distal aorta was calcified, while not more than the inner half of the media of the proximal aorta was similarly involved.

Each aortic branch had individual susceptibility to mineralization, and each had its own pattern of calcium deposition. In general, branches of similar size and structure had similar susceptibilities and patterns, but this was not an invariable rule. Without undue description of deviations, the trends in the different systems may be given as follows. As the elastic structure which dominated the composition of the upper aorta was gradually replaced by an increased amount of smooth muscle in the distal aorta and carotid, renal or iliac arteries, calcification of the internal elastic membrane became more conspicuous and local deposits of calcium appeared in and between the subjacent smooth muscle cells (Fig. 14). The deposits in the beginning were discontinuous and distributed in an annular manner (Fig. 12). As they increased in number and extent, they tended to fuse to form annular rings and to spread in the long axis of the media, eventually occupying it entirely. The process was especially conspicuous in the iliac and femoral arteries. A similar lesion but with less annular and axial discontinuity of calcification was common in the carotid, axillary and brachial arteries (Fig. 13).

Changes in the smaller and more peripheral branches of these arteries did not follow any uniform pattern which could be related to the dimension or structure of the vessel. For instance, the immediately extrinsic and intrinsic vessels of the cerebral vascular system were always normal. The main pulmonary artery and major branches occasionally contained discrete calcified lesions of the inner media, but the minor branches down to the level of the alveolar capillary network were usually normal. At the alveolar level, calcium deposition became conspicuous and extended in continuity to involve the media of vessels of the outflow venous system into the left atrium (Fig. 4). Arteries to special organs such as the submaxillary glands, kidney, spleen, thymus, duodenum, acid-secreting part of stomach and thyroid were often very severely affected, while those supplying the eye, testis, ileum, adrenal, pituitary and liver were spared from significant alteration. Arteries to skeletal muscle, pancreas, bone marrow and skin contained lesions to a variable degree among different animals and from place to place in the same animal. These general statements also pertain to arterioles and capillaries.

Veins, except those carrying blood from pulmonary alveolar spaces to the left atrium, were never electively involved, though venous channels in the kidneys, bone marrow and elsewhere were occasionally secondarily affected in connection with massive calcification of regional tissue.

Not only were there unexplained vagaries in localization of calcium in different arteries and arterial systems, but the patterns of reaction associated with the localization were equally variable. For instance, fibrous intimal proliferation was negligible in the proximal aorta. It increased in magnitude in the aorta with increasing distance from the aortic valve. It was maximal in the systemic arteries of large caliber which showed severe calcification and fragmentation of internal elastic membranes supported by medias composed largely of smooth muscle rather than layers of elastic lamellae. It was limited exclusively to areas covering abnormal internal elastic membranes and subjacent medial structures in most animals, and predominantly so in all animals. The fibrous intimal plaques formed sluggishly but at times within 6 weeks acquired a thickness equal to that of the arterial wall (Fig. 14). In smaller arteries, the intimal proliferation was less conspicuous so that in arterioles, despite extensive transmural calcification, intimal proliferation was scarcely recognizable, except in the presence of active or healed arteritis (Fig. 16).

The occurrence of arteritis was another unexplained variable. The evidence indicated that the arteritis was related to exceedingly high dosage levels and perhaps to acute intercurrent infection. Suffice it to say that arteritis of the type occurring in animals with hypervitaminosis D was never encountered in the control animals, many of which also died of acute intercurrent infections. Whatever the eventual explanation, the arteritis and periarteritis had no specific connection with the degenerative calcifying disease of arteries in general. There was good evidence that it was connected with the occurrence of calcified deposits in distal arterial systems lying within muscle and concerned principally with the nutrition of cardiac and skeletal muscle (Figs. 1, 16). Seldom was arteritis or periarteritis encountered elsewhere and then only in isolated vessels and never in the conspicuous generalized form noted in the muscles.

Not only was the anatomic distribution of the arteritis a matter of considerable significance, but the pattern of inflammation of arterial walls and perivascular tissues was equally interesting. The most severe forms were in the nature of an indolent panarteritis, which resembled reactions occurring in periarteritis nodosa, lupus erythematosus, rheumatic fever, and occasionally rheumatoid arthritis. In less severe cases,

the signs of inflammation were principally in the collagenous adventitial tissues. These signs varied from a mild nodose swelling of the fibrils to a more pronounced response characterized by aggregates of histiocytes, neutrophils and lymphocytes in and around foci of degeneration of perivascular interstitial tissues. At times, especially in the myocardium, several persons who have studied these sections have remarked the similarity of the lesions to Aschoff bodies. Similarity was all that could be claimed, however. The lesions were entirely unlike the random inflammatory foci encountered occasionally in the myocardium of rabbits in the control series.[1,8]

Muscular Systems

The preceding descriptions have disclosed that the muscular tissues of the body were usually affected. There was no evidence that this involvement was secondary to alterations of arteries, although arterial lesions customarily accompanied muscular abnormalities. Smooth muscle was more generally affected than cardiac or skeletal muscle. Changes in smooth muscle of the media of systemic arterial and pulmonary venous systems, the *tunica muscularis* of the alimentary tract and the tracheobronchial tree, and the mucosa of the renal pelvis have already been described. Similar changes were less common in such other locations as the corium of the skin and septa of the spleen. Though successive stages of development of the lesions in smooth muscle were similar in most locations, this was not true of all locations, and there were wide differences in the susceptibility of various smooth muscle cells. In general, cardiac and skeletal muscle were less susceptible than smooth muscle, whereas skeletal muscle in most locations was less susceptible than cardiac muscle. In some instances, manifestations of inflammation and cellular degeneration preceded the microscopic signs of calcification of muscle. In other instances, however, the intracellular deposition of calcium seemed to occur in muscle which showed no definite evidence of inflammation or degeneration (Fig. 6). Whether these occurred or not seemed to be related to the rapidity of development and severity of the disease. At times, the stromal elements around smooth muscle cells were affected before there was conspicuous calcification of the cells. At other times, the cells were initially altered, and the stromal elements resisted calcification. This resistance of the stroma was generally apparent when cardiac muscle was undergoing calcification, but less so when calcium was being deposited in skeletal muscle cells. In the latter instance, the sarcolemma of some fibers was calcified before the cells had acquired much calcium.

Skeletal and Hematopoietic Systems

The dominance of this disease in many tissues concerned with motion is well illustrated by the remarkable changes which occurred in the skeletal system.[12,18] The first effects occurred early and were characterized by a resorption of bone, a decrease in the prominence of osteoblasts, and an increased prominence of osteoclasts (Fig. 9). These changes were more conspicuous in cortical than trabecular structures. A significant amount of pathologic calcification of the viscera did not occur unless these alterations were demonstrable. The development of abnormal calcium deposits in the viscera was also accompanied or followed by abnormal basophilic deposits of osteoid tissue in the skeleton. These deposits occurred not only in locations notable for the degree of osseous resorption but also in the bone marrow, along the margins of persistent trabecular and cortical bone (Fig. 10). Though there was some local increase in stroma which either preceded or accompanied the abnormal massive osteoid deposits, any close resemblance to normal sequences of osteoblastic orientation, osteoid production or calcium deposition was lacking. The osteoid tissue seemed to engulf old stroma, reticulum cells and other structures in a spreading wave beginning at the margin of pre-existing bone and progressively obliterated the adjacent bone marrow. When this process ceased, the customary form of normal eosinophilic osteoid tissue with incorporated osteocytes began to appear as local islands in the midst of the widespread basophilic osteoid deposits. This indicated onset of repair and, with passage of time, the deposits were largely replaced by new bone. There resulted an osteosclerosis characterized by excessive reformation of bone along lines which did not reproduce the initial or normal pattern of bone growth. The final result was a deformed skeletal structure, usually with a porous cortex. This was accompanied by a thick layer of periosteal new bone, especially excessive at the margins of some articular surfaces, and an excess of trabecular bone which had encroached upon an abnormal bone marrow (Fig. 10). As a rule, the response of the bone marrow to calcification and osteogenesis was fairly consistent in the areas which remained free from fibrosis. The marrow which had not been replaced by fibrous tissue, abnormal osteoid tissue, or new bone, was depleted of fat cells and was densely cellular. Hematopoietic elements varied considerably in their relative proportions and stages of maturation in different animals. The commonest abnormalities were maturation arrest and diminished numbers of megakaryocytes associated with an increase of cells resembling megaloblasts or plasma cells. Conspicuous cytologic changes in the marrow were

usually accompanied by calcification of the media of blood vessels which appeared to belong to the arterial system. As yet, no connection between the changes in hematopoietic elements and the severity of the disorder in other parts of the body has been established though a correlation with gastric disease is strongly suspected.

The spleen was usually not severely disturbed. The earliest deposits of calcium occurred in the interiors of the septa. Here, fibroelastic tissue and smooth muscle were electively calcified, but the bulk of the calcium was deposited in collagen. There was no consistent evidence of any inflammatory or degenerative change prior to calcification, although collagen fibrils often were swollen and irregular in outline. In some instances the arterial system was more heavily calcified than the trabecular structure. Here, the main splenic artery and its major branches regularly showed calcification with fragmentation of the internal elastic membrane overlying foci of medial calcium deposition. Fibrous intimal proliferation was seldom conspicuous but, when found, always was superimposed on degeneration of the vascular wall. As the splenic arterial branches entered the spleen and decreased progressively in diameter, transmural calcification became increasingly conspicuous so that the walls of many follicular arterioles were uniformly calcified. Nor did the impregnation of vascular structure cease here, for in some cases it continued into the walls of the sinusoids and outlined the reticular framework of lymphoid follicles.

Endocrine and Other Glands

The principal calcium deposits in endocrine glands were in the thyroid and thymus. In the thyroid the small arteries were usually severely affected in animals with advanced generalized disease. The vascular process was associated with atrophic changes in glandular epithelium and diminution in the amount of colloid. This in turn was related to variable calcium deposits in basement membranes of follicles and interfollicular stroma.

The thymus gland showed conspicuous atrophy in severely diseased malnourished animals. Calcium deposits were restricted principally to Hassall's corpuscles although occasionally there were deposits in the media of small arteries and arterioles.

The pituitary never contained calcium deposits. There were unexplained variations in the ratios of cell types in the anterior lobe. The parathyroid glands were normal in size and in histologic characteristics. The adrenal glands were resistant to calcification with deposits occurring only in the most severe cases. These appeared only in occasional

swollen endothelial cells of the vascular sinuses in the cortex. Large vessels were spared.

The testes were seldom affected, except insofar as the illness led to reduced spermatogenesis. There were occasional calcified concretions in tubules and insignificant calcium deposits in the media of large arteries leading to the testis; these were minor late manifestations of severe generalized disease.

Other glandular structures had their individual changes. The liver was never affected, even though the arterial branches at the hilus showed the same types of calcium deposition as other branches of the celiac axis. Intrahepatic vessels were free from lesions.

The large branches of the splenic artery to the pancreas were affected to about the same extent as the arteries to the spleen. The vascular changes decreased with diminishing size of the arteries so that in contrast to splenic arterioles the arterioles in the pancreas were usually normal. In occasional lobules of the pancreas of severely affected animals, the process extended into the arterioles, and the extension was accompanied by atrophy of acinar cells with calcification of the delicate basement membranes of glands. The pancreatic islets were not affected.

The arteries and arterioles supplying the submaxillary glands were among the most susceptible peripheral vascular structures. The walls of these vessels were often densely calcified. The process varied from one lobule to another. Accompanying the most severe arteriolar lesions, there was a tendency for other degenerative and calcific changes to occur; these were epithelial atrophy, calcification of inspissated secretion in the acini of glands or small ducts, and heavy impregnation of basement membranes with calcium. The sequence of these changes was not clearly defined, but the evidence indicated that vascular and epithelial changes preceded changes in the intervening stroma.

Central Nervous System and Eye

The eye was regularly spared except for small deposits of calcium in the sclera. This comment excludes any consideration of the lens because it was not examined microscopically. Retinal arteries were never calcified.

There were no deposits of calcium in the brain or spinal cord. This was a consistent observation and applied to all structures ordinarily associated anatomically with the brain and spinal cord. The level to which calcified deposits in the carotid, vertebral and spinal arteries extended from their points of origin has not been determined, but no vascular alteration has yet been found in the intracranial or intraspinal

divisions of these vessels. Furthermore, the vascular system within the confines of the pia mater and gray or white matter was always spared from calcification even around foci indicative of chronic intercurrent meningo-encephalitis which was encountered in a few animals of our current stock.

Skin and Adipose Tissue

The skin showed changes which need not be described in detail. Beneath the epithelium and at a slightly deeper level in the corium, the lesions resembled those occurring in fibroelastic tissues elsewhere. In connection with these alterations in their advanced form, there was mild calcification of the tributary arteriolar and precapillary walls. Other histologic features resembled the early modifications in human cutaneous fibroelastic tissue designated "senile elastosis." In the deeper tissues, lesions were insignificant except where short stretches of fascia were lightly calcified and where skeletal muscle showed degeneration of the type described elsewhere.

Adipose tissue in most locations was unaffected by the disorder. However, in the retroperitoneal tissues, especially around the larger arteries and adrenals, severe atrophy of fat cells was encountered, usually accompanied by a deposition of calcium. The principal deposits were either adjacent to or in the cytoplasmic membranes of fat cells. No massive deposits were encountered in the interior of fat cells.

Discussion

A major problem in analysis of the pathogenesis of many generalized diseases is an inability to explain the vagaries of distribution of the anatomic or functional manifestations. In one patient the manifestations of a disease may be referable to a single system or some part thereof. In another, the manifestations of the same disease may be referable to more than one system or more than one part of any system. In still others, the manifestations may be dissociated so that the expected quantitative relations between the anatomic and functional changes are not found. Concepts which guide thinking in these matters have no solid foundation in structural or functional pathology.

It was our belief that an experimental study of an easily controlled metabolic derangement which produced widespread structural changes of a simple degenerative nature rather than a complex inflammatory reaction might lead to formulation of more suitable concepts.[19] Wolbach demonstrated with well-planned experiments and great interpretive insight that the use of deficiencies and excesses of vitamins was an elegant method for approaching problems of this kind.[16-18] After surveying this subject, it was decided that a systematic experimental

study of hypervitaminosis D might be helpful for our purposes, and that this eventually might be compared with experimental hyperparathyroidism.[11,21,22]

The widespread changes disclosed by this study may be summarized as follows. First, degenerative changes with calcium deposition occurred in many organs and tissues. Second, the pattern of distribution of the degenerative changes was not specifically related to any common physical, chemical, or other characteristic of the affected elementary tissues or their combinations. Third, the sequences in development of these changes were not the same everywhere but were determined in part by unknown factors characteristic of the affected organ or tissue. Finally, the pattern and sequences of degeneration occurred in a reproducible fashion which could not have been predicted from a knowledge of factors which ordinarily determine the local characteristics of generalized disease processes.[19]

The widespread distribution of pathologic changes was largely dependent upon the amount of irradiated ergosterol given, the interval between doses, and the duration of the experiment. The data indicated that a sufficient quantity of the vitamin would produce minimal changes if it were given in a single dose or in a series of small daily doses over a period of several days. Maximal subacute changes due to the same quantity of the vitamin occurred when it was given in equal divided doses at intervals of 2 or 3 days. The best regime for obtaining maximal, chronic, slowly progressive changes has not been established, but good results followed the interposition of prolonged rest periods between brief dosage schedules designed to produce bursts of maximal active changes.

The pattern of distribution of pathologic alterations was constant under a given regime but varied somewhat with variation of the experimental conditions. In general, however, the earliest evidence of degeneration appeared in a particular location in each affected tissue or organ. Then, the process spread to involve other tissues or the same tissue in a succession of locations, each of which was resistant to change until the antecedent pattern of degeneration was set. Each affected type of tissue in each affected location in each affected organ had a definite level of susceptibility to the degenerative changes. This curious order of susceptibility to degeneration with calcification led to the conclusion that each type of cell or tissue in a functionally integrated system had a characteristic degeneration-calcification potential and that the level of the potential varied among different integrated systems. Hence, the succession of tissue alterations might be regarded as a result of the operation of four mechanisms. The first mechanism

raised the calcification potential of the tissue. The second raised the calcifying potential of the environmental fluids. The third was concerned with adaptation, and the fourth with restoration.

It is generally accepted that the best way to increase the calcification potential of a cell or tissue which does not normally calcify is to reduce its viability without bringing about rapid structural disintegration.[11] In the present experiments, it was clear that there was a reduction in viability of certain cells and stromal elements. It was not clear whether the reduction preceded or followed the deposition of calcium salts.[8] If reduction in viability preceded calcification, there was at this stage little evidence of customary findings of lessened viability. In other words, the earliest evidence of modification of stroma or cells did not definitely precede evidence of calcification in many locations. Furthermore, in most pathologic conditions, the reduction of viability of stroma and cells ordinarily initiates a sequence of inflammatory and regenerative reactions before calcium deposition becomes conspicuous. In the present study, this was not encountered as an early reaction in any location, except perhaps in certain muscular tissues, and then only in connection either with intolerably high dosage or intercurrent infection. However, under these conditions, conspicuous inflammatory reactions did not occur prior to degenerative calcifying changes, and the reactions were restricted principally to the arterial systems in cardiac and skeletal muscle. Usually, the reactions were confined to the media and adventitia of arteries, but in the severe cases there was a spread into neighboring muscle. The distribution of the inflammatory reaction, when it occurred, coincided with the distribution of the degenerative calcifying changes which developed without signs of inflammation in muscle of other experimental animals. From this it may be inferred that some factor increased the degeneration-calcification potential in all instances, but an inflammatory reaction was not elicited except when it was excessive or when other factors, perhaps related to intercurrent infection, were introduced. In any event we must admit the possibility of a more complex form of local tissue potential which might be called an inflammation-degeneration-calcification potential. Thus, under the same systemic conditions local factors may induce various combinations of inflammation, degeneration and calcification to occur at the same time in different organs or in the same tissue types in different organs.

The second mechanism seemed to be related to the composition of oxygenated or arterial blood and to special units of tissue integrated together in the performance of some particular function. For instance, emphasis has often been placed upon the deposition of calcium in tis-

sues concerned with acid secretion.[2,5,11] The explanation has been that the secretion of acid led to a local alkalosis which in turn enhanced the likelihood of local precipitation of calcium phosphate and carbonate. Inasmuch as arterial blood is ordinarily more alkaline than venous blood, the view may be taken that through diffusion, systemic arterial walls may be more alkaline than venous walls, the reverse being true in the pulmonary system. The view may also be taken that the alkalinity of oxygenated systemic arterial blood decreases progressively with increasing distance from the alveolar capillaries and venules. Hence, if the interstitial fluids of the vascular wall reflect the local alkalinity of the blood, the deposition of calcium and associated degenerative changes of constituent tissues might be expected to fall off progressively in the direction of flow of the arterial blood. The microscopic observations disclosed that this was not always so. In a further analysis of this matter, the lung, kidney and stomach may be considered separately as the three principal acid-secreting organs.

Theoretically, it might be predicted that the region of maximal alkalinity in the lung would be in connection with the blood in the postalveolar pulmonary venules, and that maximal degenerative calcifying changes would occur in the walls of these vessels. In general, this was the case (Fig. 4). There were negligible lesions in the pulmonary arterial system, and the conspicuous changes were initially found in the walls of interalveolar capillaries and adjacent interalveolar stroma. Later, the venules and small pulmonary veins were affected. Insofar as the respiratory passages are concerned, if we accept free diffusion of gases into tissues, the level of maximum alkalinity would be in the upper trachea (Fig. 3). Hence, it might be predicted that the changes would be more conspicuous here than in the terminal bronchi. In general, this was found. The tracheal structures showed the earliest changes, and as a rule calcification along the respiratory passages decreased progressively in identical tissue types in identical locations with increasing distance from the larynx. In a consideration of this rule, however, other factors have to be considered. First, the lesions around the circumference of any respiratory passage were not uniform at a given level. The earliest changes were either in goblet cells of the epithelium or in the subjacent stroma. With increasing time and severity of the disease, the changes spread circumferentially and also in a very specific way in depth. The circumferential spread finally led to involvement of the entire basement membrane zone and an increasing span of epithelium. The spread in depth tended to skip the loose collagenous tissues of the mucosa and to appear in the walls of mucosal vessels nearest the subepithelial zone of calcification, the smooth

muscle in the neighborhood, and the part of bronchial cartilages contiguous to the maximal mucosal changes. Thus, it seemed that the distribution, the sequences, and the magnitude of lesions were determined by the operation of local influences, as follows: alkalinity of environmental gases and fluids, type of tissue, type of cell, and the locally integrated functions of susceptible types of tissues or cells.

An analysis of alterations in the stomach may also be attempted in terms of relative alkalinity of parts of an acid-secreting organ. One might postulate that maximal alkalinity would be in relation to the venous blood flowing from the acid-secreting mucosa and that maximal calcification would occur in the tissues supporting the acid-secreting glands and in the walls of vessels leading from these tissues. This was not found even though, when mucosal changes did occur, they were restricted to the acid-secreting mucosa and to the *tunica propria* or cells of glands responsible for this function. Such changes, however, were inconspicuous in the early stages and often followed the development of prominent changes in the walls of arteries, the *tunica muscularis,* and muscularis mucosae (Fig. 5). Veins or venules were seldom affected. It might be assumed, therefore, that if relative alkalinity governed the distribution of the lesions in the stomach, it would have to operate in a curious way throughout the gastric wall. Consequently, it seemed that an inquiry should be made into the role of locally integrated functions of susceptible types of tissues or cells. It was apparent that the calcification was not a disorder of the acid-secreting gastric mucosa alone but a disturbance of the full thickness of the gastric wall concerned with acid secretion. For present purposes, the acid-secreting part of the stomach should be considered transmurally as a functional unit, separate and distinct, pathologically, from the remainder of the stomach. We have no explanation as to why the arterial system, the *tunica muscularis,* the muscularis mucosae and, to a lesser degree, the mucosa of only this part of the stomach were so severely affected by this degenerative calcifying disease. It offered a puzzling example of localization of pathologic changes in all susceptible tissue types in a functionally integrated system in a special location. The remainder of the intestinal tract offered other problems in this connection; namely, the variable pattern of involvement of the *tunica muscularis* at different levels, the variable resistance of different mucosal types to the disorder, and the restriction of severe arteriolar disease to the duodenal and, to a lesser extent, colonic segment of the intestinal tract.

Changes of the type found in the kidney have also been attributed to a local alkalosis, secondary to secretion of acids by the kidney.[10,11] It might be predicted therefore that changes would occur in that part

of the kidney just distal to the postabsorptive tubulovascular structure or in some special region concerned with selective absorption of alkaline elements or selective secretion of acidic elements. Actually, the earliest alterations occurred in the cells of the proximal convoluted tubules, soon thereafter in the basement membranes of these tubules, and somewhat later in the arterial supply to the affected nephron rather than the venous return from the nephron (Fig. 7). Thereafter, the arterial tree was usually progressively involved with increasing severity (Figs. 8, 15). It was concluded that within the limits of present knowledge of renal function, other factors than local alkalinity determined the distribution and sequence of the changes. As in the instance of the lungs and stomach, these factors were concerned with the interrelationships between susceptible tissue types in a functionally integrated system in a given location. The spread of changes in the kidney demonstrated this very well. In the early stages only a scattering of total nephrons as complete units showed lesions, but these were the nephrons with short arterial connections close to the medullocortical junction. As more lesions developed, the intervening nephrons with similar arterial connections were affected. With increasing time and severity of the disease, there was a spread of the changes distally toward the capsule, but seldom did the animal live long enough to show much alteration in the nephrons of the peripheral third of the cortex. As cortical spread developed, there was a similar spread of changes in an ever-broadening medullary zone within which the medullary loops of the successively affected nephrons were presumably located. These sequences are presumably related to the distribution of functional load rather than of a specific secretory function among a system of nephrons.

The preceding comments have touched upon local factors such as alkalinity, type of tissue, type of cell and locally integrated functions of susceptible tissues or cells. Muscle cells were among the least resistant, the order of decreasing susceptibility being smooth, cardiac, and skeletal muscle. Furthermore, the most readily affected component of these cells was the contractile protein, especially the "A" disk. Among the stromal elements, the severest lesions appeared in elastic tissue, basement membranes, the matrix of cartilage and the sarcolemmal investiture of muscle cells. Cellular and stromal elements were not equally susceptible in all locations. Hence, the development of this systemic metabolic disorder did not result in a generalized modification of a given element, and this is analagous to the effects of certain "toxins."[23] On the contrary, it led to conspicuous modifications of these elements in specific locations without changes in identical struc-

tures in other locations. The extent to which this selectivity may be attributed to unrecognized differences in elements assumed to be identical, remains an intriguing problem. Certainly there are good reasons for believing that the composition of structures such as smooth muscle cells, collagen, elastic tissue, basement membranes and cartilaginous matrices is not the same everywhere. By the same token, the functional burden to which these elements are subjected is not the same everywhere.

The third mechanism was concerned with adaptation. Three adaptive activities deserve mention: the intimal proliferative reactions in arteries; the excessive production of atypical osteoid matrix following extensive resorption of bone; and the regression of the disease despite continuation of the same dosage regime which produced it. The discussion of these and other less obvious adaptive reactions awaits the completion of more prolonged experiments.

The fourth mechanism was concerned with restoration, principally the resorption or elimination of abnormal mineralized tissue, and the regeneration of tissues to replace those attacked by the disease. The salient feature of this mechanism was its indolence. In the respiratory mucosa, desquamation of calcified goblet cells and regeneration of basal cells to replace them were sluggish processes. At times, the regenerative sequences took a metaplastic trend so that the degenerated epithelial layer was replaced by one or more layers of stratified squamous epithelium. Wherever this occurred, there were local defects in the basement membrane and chronic inflammation in the subjacent mucosa. The mineralized basement membranes also tended to persist, being very slowly resorbed (Fig. 3). With increasing mineralization, there was increased fragility of these structures. Fractures developed and discontinuities thereby created seemed to stimulate local proliferation and accumulation of histiocytes. These commonly formed multinucleated giant cells which slowly resorbed the mineralized stromal structure, often encapsulating it completely. As this proceeded, the mineralized basement membrane seemed to migrate to a deeper level, and a regenerated stromal support appeared as a new structure between it and the respiratory epithelium. Similar mechanisms of encapsulation and resorption were encountered as far distally as the alveolar ducts but were seldom detected in connection with the calcified components of alveolar walls or pulmonary vascular channels. Alveolar walls seemed to break down spontaneously with the production of emphysema, but it was not clear that these mechanisms participated in the rupture (Fig. 4).

The behavior of the mesenchyme adjacent to abnormally mineralized

cartilages along respiratory passages was also interesting. Intense mineralization was usually conspicuous on the mucosal side of the cartilage. The perichondrium became less distinct, and the margin of the calcified matrix acquired a moth-eaten appearance. Capillaries occasionally invaded the marginal lacunae, and in some instances there was active formation of new cartilaginous matrix by proliferating chondrocytes. These activities resembled those which normally occur at provisional zones of endochondral bone formation. The overall result here, however, was the slow resorption of the heavily calcified bronchial and tracheal cartilages without osteogenesis.

Resorption and regeneration were still more sluggish in the kidney. Calcified tubulovascular components of the nephron seemed to endure without resorption or regeneration for at least several weeks. Evidence of regeneration of tubular epithelial cells of affected nephrons, compensatory hyperplasia of tubules of spared nephrons, interstitial fibrosis, inflammation, arteritis, glomerulosclerosis or other responses were never significant. The principal reaction occurred in the precortical and large cortical arteries. Here, there was intimal proliferation, excited less by degeneration and calcification of the media than by discontinuities due to fractures of calcified internal elastic membranes (Fig. 15). Whatever lesser reactions occurred in the subepithelial stroma of the renal pelvis were similar in most respects to those described in connection with the basement membranes of the respiratory tract.

Resorption and regeneration in relation to the acid-secreting part of the gastric wall were also very sluggish. The mucosal structures were not very responsive to the degenerative changes. The intimal reactions in the arterial tree were more active than in other organ systems, and the proliferative reactions also extended further into the smaller arterioles. The muscularis mucosae and the *tunica muscularis* showed the most conspicuous evidence of resorption and regeneration. In both locations, the degenerated calcified smooth muscle and intervening stroma often stimulated local histiocytic and giant cell reactions. These cells assisted in the resorptive process. Also, at times, there were many large pale elongated immature elements which appeared to be regenerating smooth muscle cells. In no case, however, was there significant leukocytic infiltration, local vascularizing reaction or fibrosis.

The complexities of resorption and regeneration in the skeletal system were difficult to interpret. Initially, the resorption of bone was accelerated, the number of osteoclasts was increased, and the number

of osteoblasts was decreased (Fig. 9). A curious proliferative process followed, resulting in the formation of a large amount of abnormal osteoid matrix beneath the periosteum and in the marrow around the residual cortical and trabecular bone (Fig. 10).[12,13] Though this matrix may be called "osteoid," it was more fibrillar, less homogeneous and much more heavily stained with hematoxylin than normal osteoid tissue. Furthermore, there was no relation between the voluminous deposits of matrix and any particular array of cells which resembled osteoblasts. The tissue looked more like collagen which had as much affinity for hematoxylin as any of the extra-osseous calcified stromal or cellular elements. With onset of repair, normal eosinophilic osteoid tissue and bone appeared in the midst of this voluminous basophilic fibrillary material which was resorbed. It was conspicuous only when extra-osseous calcium deposition had essentially ceased or in the period following termination of ergosterol administration. The prompt and rapid formation of bone was more pronounced in connection with the peritrabecular matrix and in the subperiosteal regions adjacent to the margins of articular cartilages. This led to a deformed osseous framework with depleted porous cortical bone, an excess of periosteal new bone, an excess of endosteal bone, and a coarse dense trabecular structure displacing the marrow. The final result had some resemblance to osteosclerosis of a pagetoid type with myelofibrosis (Fig. 10). The development of atypical hematopoietic elements as these changes encroached upon the bone marrow is beyond the limits of this discussion.

Finally, it was clear that a moderate dosage regime was most effective in the first few weeks. As the regime was continued, the expected progression of pathologic changes failed to occur, and evidence of the disease slowly disappeared. This was attributed to the activation of obscure mechanisms which provided increasing tolerance, resistance or immunity to the action of excessive vitamin D. In the light of certain degenerative calcifying human diseases, an inquiry into the nature of these adaptive and restorative mechanisms would seem to be of first importance.

Summary

A microscopic study of the evolution of hypervitaminosis D in rabbits disclosed generalized pathologic sequences which were due to partly reversible inflammatory, degenerative and mineralizing processes. The participation of these processes in the pathogenesis of the disease in a given location was governed principally by the level and duration of dosage with the vitamin. Administration of the vitamin in amounts just sufficient to produce pathologic changes in 2 or 3 weeks

led to mineralization of certain tissues which do not normally calcify. Under these conditions the deposition of calcium occurred without significant preliminary local structural modifications attributable to inflammatory or degenerative processes. When somewhat larger doses of the vitamin were used, abnormal mineralization of tissues was often preceded and accompanied by structural changes of a degenerative type. In order to excite significant early inflammatory reactions, still larger doses of the vitamin and perhaps other factors were required. These reactions occurred only in cardiac and skeletal muscle. They were localized to the walls of arteries, periarterial tissues and adjacent muscle, in which there were signs of progression of degeneration and mineralization.

Studies of restorative phases of the disorder disclosed evidence of reversibility and resistance to progression of these processes. The inflammatory reactions in muscle subsided, and mild indolent reparative reactions appeared in many degenerated mineralized tissues. Furthermore, additional degenerative changes seemed to progress very slowly, if at all, while, at least in some locations, the process of mineralization was slowly reversed even though the dosage was maintained. The reversal was characterized by the resorption of abnormally calcified matrices. At times, this occurred spontaneously, but as a rule the resorption was associated either with mobilization of macrophages and giant cells or penetration of the calcium deposits by vascularized stroma. The signs of reversal were usually preceded or accompanied by resumption of osteogenesis in the abnormal osteoid matrices in bone.

The same type of tissue or cell in different locations was not equally affected by the inflammatory-degenerative-mineralization mechanisms, presumably because of a difference not only in the composition of tissue gases and fluids from place to place but also in the susceptibility of a particular tissue in different locations. The latter was governed primarily by participation of a cell or tissue in a functionally integrated and spatially related system which had in one or more of its parts a high calcification potential.

The designation of function as adding to or detracting from the inflammatory-degenerative-calcification potential of a given structure led to a better understanding of patterns of the disease, especially in the respiratory tract, kidney, stomach, muscles and arteries. It may also assist in the future analysis of the reasons for the resemblance between certain structural changes in this experimental disease and those encountered in some presumably unrelated human disorders which are indolent, fundamentally degenerative, and ordinarily attributed to ill-defined metabolic derangements of advancing age.

Among these, senile osteoporosis, hypertrophic arthritis, pulmonary emphysema and arteriosclerosis deserve more than fleeting consideration, so long as it is recognized that identity of structural change does not imply identity of pathogenesis.

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LEGENDS FOR FIGURES

FIG. 1. Multiple, focal, inflammatory-degenerative-calcification changes in the myocardium of a rabbit given 600,000 units of viosterol in 2 weeks. The inflammatory reaction is located principally in and around the walls of small arteries and arterioles which in turn are surrounded by cardiac muscle showing degeneration with early calcification. Hematoxylin and eosin stain. × 200.

FIG. 2. Massive calcification of cardiac muscle in a rabbit given 1,200,000 units of viosterol in 7 weeks. The degenerated calcified muscle cells have multiple transverse fractures through which proliferating stromal elements have grown. The isolated calcified fragments of cells have undergone partial resorption. There is no inflammatory reaction. Hematoxylin and eosin stain. × 150.

FIG. 3. The tracheal mucosa of a rabbit given 1,200,000 units of viosterol in 7 weeks. Intra-epithelial calcification is in the form of concentrically laminated pseudocrystals which seem to develop exclusively in the goblet cells. The subepithelial calcification is concentrated in the darkly stained fragmented basement membrane. Note the absence of inflammation. Hematoxylin and eosin stain. × 500.

FIG. 4. A peripheral field of the lung of a rabbit given 1,200,000 units of viosterol in 6 weeks. Hematoxylin has a strong affinity for calcified tissue, demonstrated here as deeply-stained black segments of an emphysematous alveolar wall and the entire thickness of the wall of a small pulmonary vein. These changes in alveolar and venous walls regularly occurred together and were independent of inflammation. Hematoxylin and eosin stain. × 500.

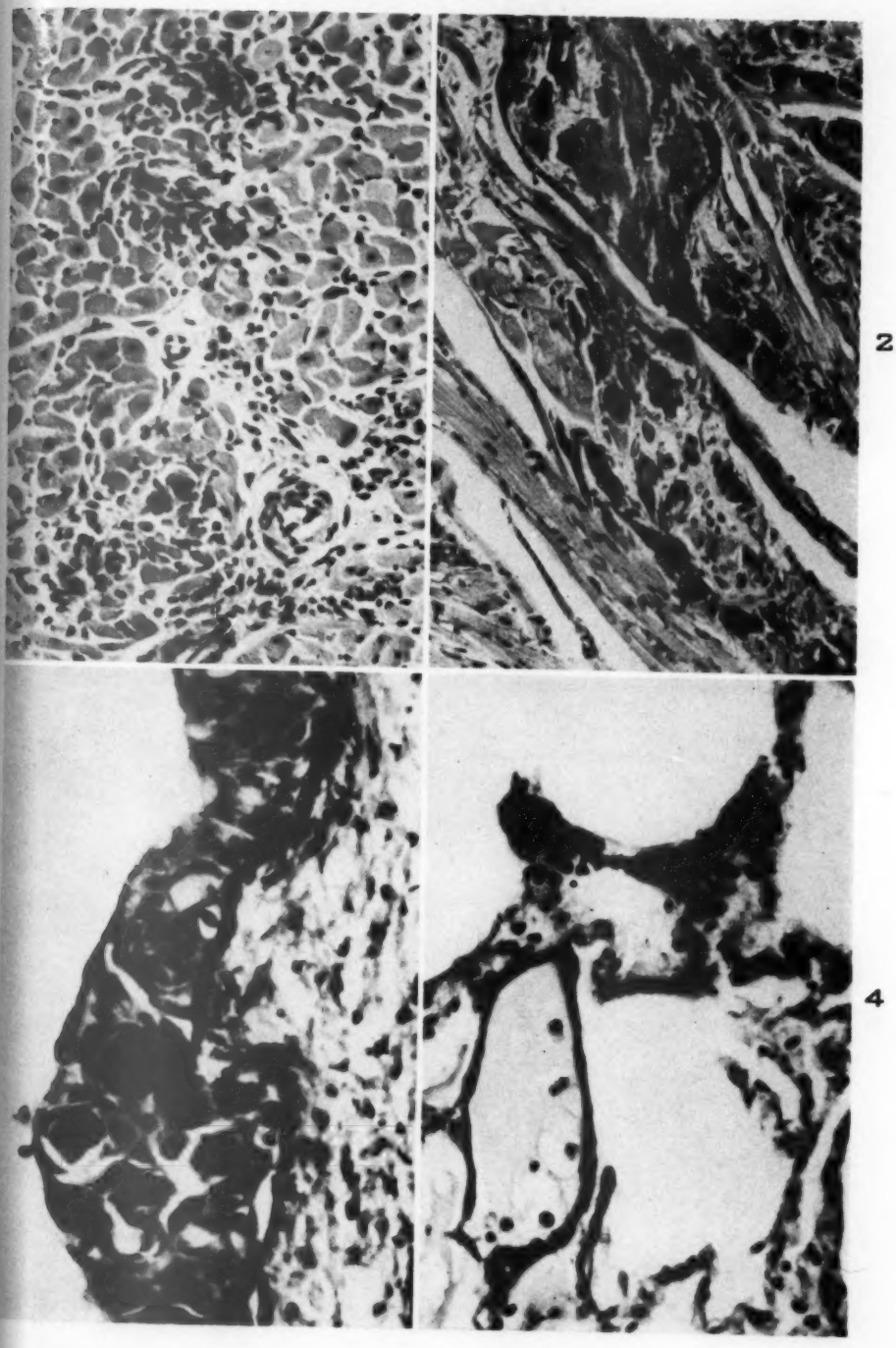
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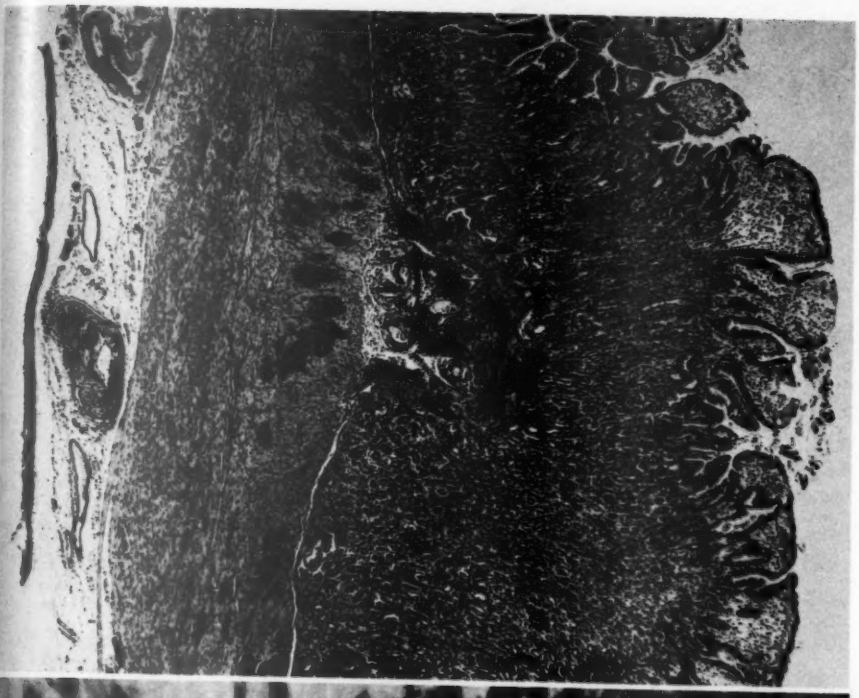
FIG. 5. A cross-section of the wall of a part of the stomach concerned with secretion of hydrochloric acid. The rabbit from which this section was made was given 1,600,000 units of viosterol in 14 weeks. Hematoxylin has an affinity for calcified tissues, shown as irregular small dark areas in the mucosa, in the walls of submucosal arteries, in the bundles of smooth muscle of the inner half of the *tunica muscularis* and in the wall of a small serosal gastric artery. Hematoxylin and eosin stain. × 40.

FIG. 6. A high-power photomicrograph of one of the small darkly stained calcified areas in the *tunica muscularis* of the wall of the stomach shown in Figure 5, showing degenerated smooth muscle cells in the midst of granules and filaments of calcified protoplasm demonstrated by its strong affinity for hematoxylin. The small calcified granules are in the cytoplasm of the muscle cells. The tortuous calcified filaments are external to smooth muscle cells and seem to consist largely of interstitial fibrous or reticular elements. Hematoxylin and eosin stain. × 600.



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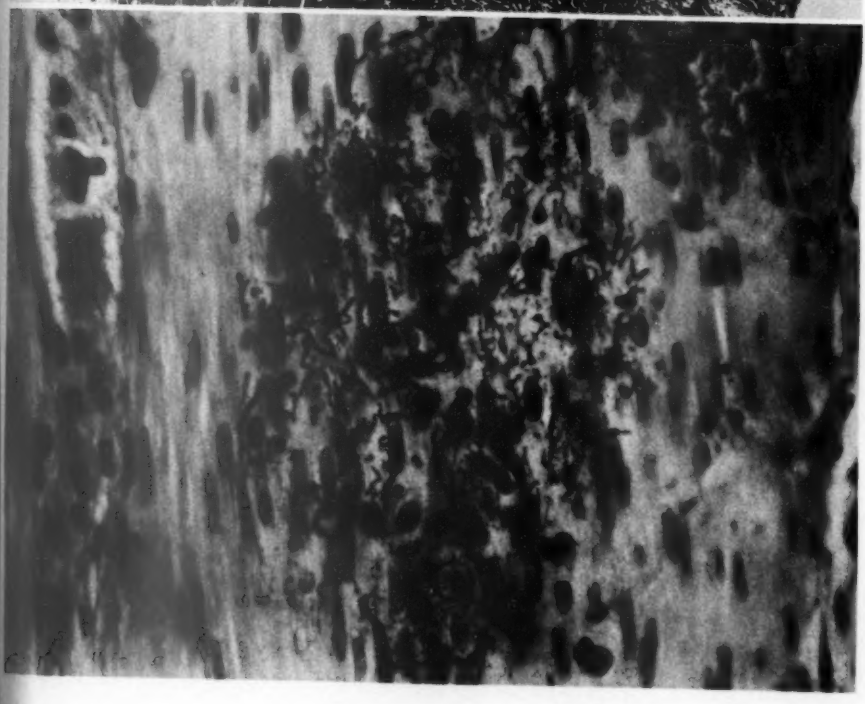

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FIG. 7. The cortex and medulla of a kidney from a rabbit given 1,000,000 units of viosterol in 5 weeks. Six weeks later the animal was sacrificed. The loss of normal structural detail is apparent in the inner half of the cortex and in a narrow transverse zone in the middle of the medulla. The small dark spots represent calcified structures in these 2 locations. The intervening lightly stained areas which are more conspicuous in the cortex are places where proliferating connective tissue has replaced degenerated tubulovascular units of nephrons. This represents an early stage of resorption of mineralized tissue with repair and regeneration. Inflammation is negligible in the kidney at all times during the development or regression of the disease. Hematoxylin and eosin stain. × 23.

FIG. 8. A field in the inner third of the cortex of a kidney of a rabbit given 800,000 units of viosterol in 4 weeks. This shows early calcification of glomerular structure with minimal associated alterations in tubular structure. The calcified elements have an affinity for hematoxylin. There is a tendency for calcium to accumulate in isolated glomerular loops. In one glomerulus the accumulation is in continuity with the calcified wall of the arteriole entering the glomerulus. Hematoxylin and eosin stain. × 220.

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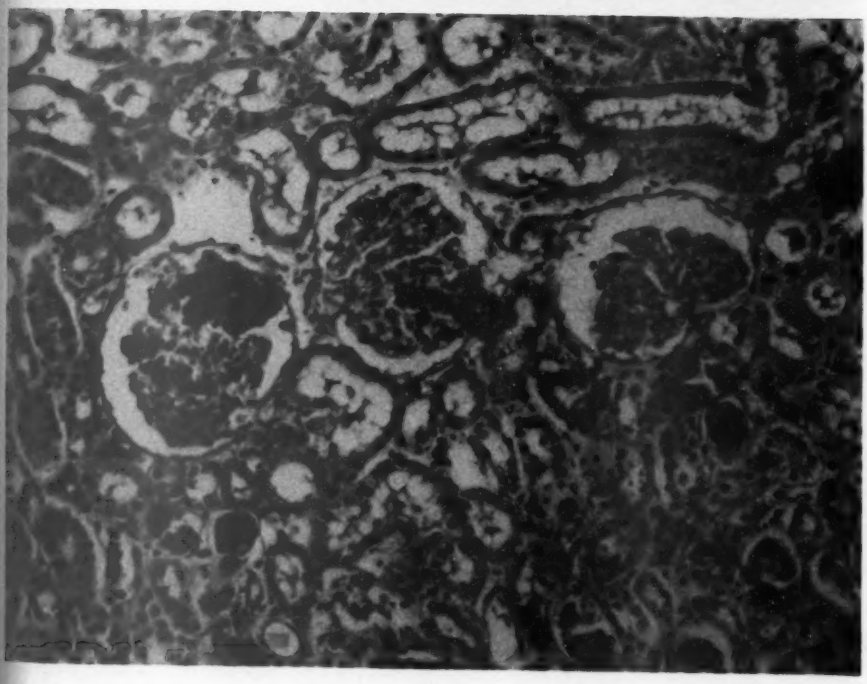

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FIG. 9. A longitudinal section of a rib from a rabbit given 1,200,000 units of viosterol in 4 weeks, showing widespread resorption of bone with conspicuous porosity of cortical bone, fibrosis of the marrow, and early subperiosteal deposition of osteoid tissue. The changes are characteristic of the disorder between the third and fifth weeks. After about the fifth week, the changes illustrated in Figure 10 become increasingly dominant. Hematoxylin and eosin stain. × 60.

FIG. 10. A longitudinal section of the vertebral column of a rabbit given 1,200,000 units of viosterol in 12 weeks. The resorption of bone, typical of early stages of the disease (see Fig. 9), has led to partial collapse of a vertebral body which is at this later stage undergoing an osteosclerotic pagetoid change. This is characterized by excessive deposition of osteoid tissue which has a conspicuous dark color due to its affinity for hematoxylin. Much of this abnormal osteoid tissue has been converted to bone, producing a dense trabecular structure which has seriously encroached upon the marrow. Also, note the proliferative changes which have produced "spurs" of new bone at the margins of the intervertebral disks. Hematoxylin and eosin stain. × 10.

FIG. 11. A cross-section of the wall of the thoracic aorta of a rabbit given 600,000 units of viosterol in 2 weeks. The inner third of the elastic tissue framework, stained deeply with hematoxylin, is uniformly impregnated with calcium. Note the absence of any inflammatory reaction though only a trace of residual cellular structure remains between the calcified elastic lamellae. Hematoxylin and eosin stain. × 250.

FIG. 12. A cross-section of an iliac artery of a rabbit given 1,000,000 units of viosterol in 5 weeks. The segmental darkly stained areas represent subintimal bands of calcific deposits in the inner half of the media. These discontinuous deposits tend to spread in all directions until they occupy the entire media. Meanwhile, there is a reactive proliferation of the intima which in time tends, as shown, to be equal in thickness to the part of the subjacent media inactivated by calcification. Hematoxylin and eosin stain. × 30.

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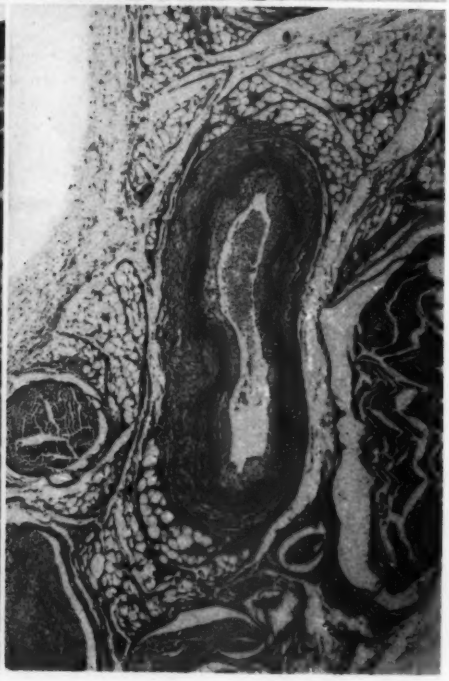

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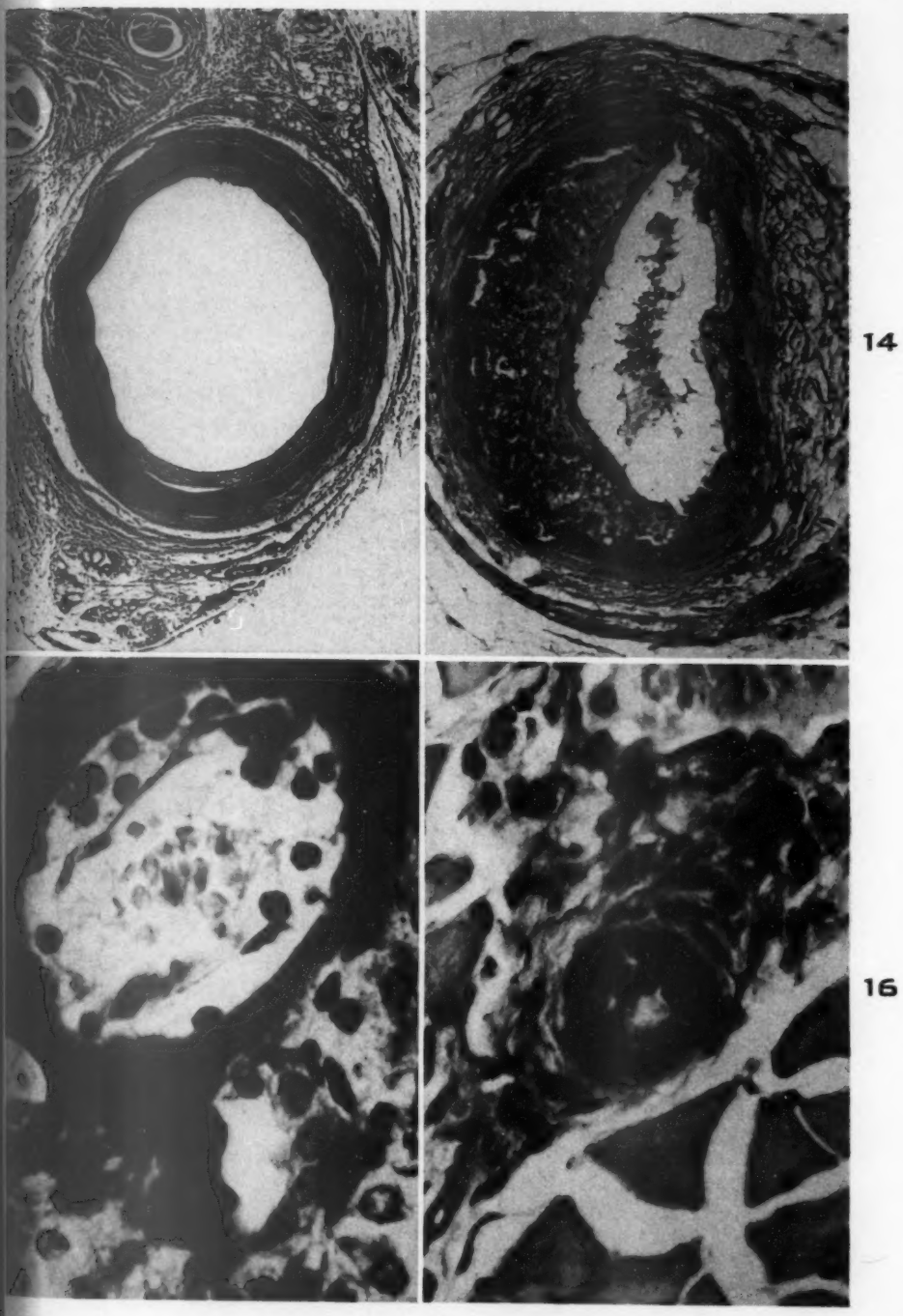
FIG. 13. A cross-section of a carotid artery of a rabbit given 1,000,000 units of viosterol in 15 weeks. From the lumen outwards 3 distinct zones are shown. They are of almost equal thickness. The inner zone is composed of proliferating fibrocellular intimal tissue. The middle zone, deeply stained with hematoxylin, is the inner half of the media which has been uniformly impregnated with calcium. The outermost zone is the external half of the media which has been unaffected by the disease. Hematoxylin and eosin stain. × 32.

FIG. 14. A pancreatic artery of a rabbit given 1,600,000 units of viosterol in 14 weeks. The darkly-stained material in the vascular wall is calcified tissue with a strong affinity for hematoxylin. The deposits of calcium involve most of the internal elastic membrane and a modest amount of the media subjacent to the calcified parts of the internal elastic membrane. Three short stretches of the internal elastic membrane and adjacent media are normal. The loose-textured fibrocellular tissue internal to the calcific deposits is proliferating intima. Note that intimal proliferation did not occur over the 3 stretches of internal elastic membrane which were unaffected by the calcific disease. Hematoxylin and eosin stain. × 225.

FIG. 15. A small artery and adjacent arteriole in the renal cortex of a rabbit given 1,500,000 units of viosterol in 2 weeks. The walls of these vessels are heavily calcified as indicated by the strong affinity of the media for hematoxylin. Swollen intimal cells with their nuclei projected away from the calcified media toward the lumen of the larger artery represent the earliest intimal reaction to degenerative calcifying medial disease. Hematoxylin and eosin stain. × 600.

FIG. 16. A tiny arteriole in skeletal muscle of a rabbit given 1,200,000 units of viosterol in 7 weeks. The media of the arteriole, stained deeply with hematoxylin, is uniformly calcified. The swelling of the intima has reduced the size of the lumen. In the adventitia there are a few inflammatory cells representing a mild form of periarteritis often found in cardiac and skeletal muscle of rabbits dying of pneumonia during the course of a high-dosage viosterol regime. Hematoxylin and eosin stain. × 500.



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16

THE EFFECTS OF PITUITARY STALK SECTION IN THE GOAT *

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In recent years there has been renewed interest in the possibility that the surgical removal of endocrine glands could be used as a means of controlling certain types of carcinoma. Some of the more promising results obtained have been those derived from hypophysectomy.[1-3] The removal of the pituitary gland, however, is a major neurosurgical undertaking, and it seemed to us that, on theoretical grounds, the considerably simpler operation of cutting the pituitary stalk would be almost equally effective in depressing pituitary function. By this procedure the anterior lobe (*pars distalis*) would be deprived of the greater part of its blood supply and of all direct influence from the hypothalamus, and the nerve supply to the posterior or neural lobe (infundibular process) interrupted. A few operations of stalk section, for advanced cancer in man, have recently been reported by Davies and Buxton.[4]

Although the effects of pituitary stalk section have been studied by many workers in various animal species, the investigations in general have not been directed primarily to the effects produced on the pituitary gland itself, distal to the lesion. Observation of these effects was the main objective in the present study. If, however, one is to be justified in applying to man the effects of pituitary stalk section observed in an experimental animal, it is essential that the animal used have a pituitary gland strictly comparable with the human pituitary, particularly in regard to its blood supply. In this respect the rabbit, in which the effects of stalk section have been studied by several workers,[5-11] is not very suitable, since the anterior lobe of this animal's pituitary, unlike that of man, is supplied by an artery as well as by the portal vessels from the stalk. Moreover, this artery is not interfered with by stalk section.[12,13] In rats, sheep and goats, however, there is no arterial supply to the anterior pituitary, and the vascular anatomy of the gland is on the whole very comparable with that obtaining in the human pituitary.

In earlier experiments we found that infarction of the anterior lobe occurred regularly in rats and in sheep after cauterization or section of the pituitary stalk.[14-16] The experiments on goats, reported here, en-

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abled us to extend our observations on the effect of stalk section to another suitable species. Also, since we allowed some animals to survive for longer periods, it permitted us to investigate the effects upon the posterior and intermediate lobes of the pituitary gland and upon other tissues of the body.

METHODS

Operated Animals

Thirteen male goats (pure-bred British Saanen) were operated on for section of the pituitary stalk. Four of these animals were 8 months old, and the remainder ranged in age from 7½ to 11½ weeks on the day of operation. One goat of the latter group died during the operation, and the pituitary glands and other organs taken from this animal, from an unoperated male goat aged about 11 weeks, and from 2 unoperated male goats aged about 8 months, were used for histologic control purposes.

The animals were anesthetized initially with pentobarbital sodium (Veterinary Nembutal;® Abbott), given percutaneously into the external jugular vein, and subsequently, as required, through a cannula inserted into the saphenous vein. A soft plastic tube of suitable diameter was passed into the trachea, a stylet consisting of a double length of copper wire being used to stiffen the tube and bend its tip for the actual passing. After the head of the goat had been shaved and cleaned, a midline incision about 10 cm. long was made in the scalp. At right angles to this a second incision was made, passing to the left, midway between the base of the ear and the orbital margin. Bleeding from the soft tissues was controlled by cautery and occasionally by ligature, and from the bone by the use of bone wax. The origin of the left temporal muscle was detached from the skull, and much of this muscle was removed. After the periosteum had been stripped off, a burr hole was made in the skull, and the calvarium was nibbled away over a wide area on both sides of the midline, but particularly on the left side where the bone was removed to the level of the base of the skull. Care was taken to avoid damaging the superior longitudinal sinus. The dura mater was then cut away, again on both sides of the midline. The goat was next laid on its right side and the head tilted still further over to the right to allow the brain to fall away from the base of the skull. In this position minimal retraction of the brain was needed to bring into view the left internal carotid artery emerging from the dura and, just rostral and medial to it, the pituitary stalk.

A high intensity lamp and, on occasion, magnifying spectacles were used to improve the operator's vision. Cerebrospinal fluid and any blood were removed by suction, and when the field of view was dry, the

stalk was severed by means of a small hook passed around it. Bleeding caused by this maneuver was usually minimal. Next, a piece of waxed paper (approximately 10 by 8 mm.) was inserted from behind forwards, between the cut ends of the stalk, the aim being to position this "wax plate" so that its front edge rested on the *tuberculum sellae*, touching the optic chiasma, and the back edge covered the *dorsum sellae*. To pass the wax plate under the internal carotid artery, it was sometimes necessary to cut the third nerve on the side of approach. No attempt was made to replace the dura or skull cap, and the skin edges were merely brought together over the exposed brain and sutured. The animals were given a mixture of penicillin and streptomycin (Seclomycin; Glaxo Laboratories, Ltd.) intramuscularly during the operation and on each of the next 3 days. No hormone replacement therapy was given either pre- or postoperatively.

All the wounds healed by first intention, and the sutures were removed one week after the operation. By this time the edema present during the first day or two had subsided, and thereafter the only abnormality seen was a depression at the site of the opening in the cranium. After spending 2 or 3 days in a well-strawed, covered pen, where the goats were kept in pairs, the animals were given access to an open yard in the daytime where they could run about freely, and were confined to a pen only at night.

The animals were sacrificed by an overdose of intravenous pentobarbital sodium, at times ranging from 25 hours to 29 days after operation, and a complete necropsy examination was carried out immediately. The organs were weighed, and selected pieces (with the exception of the pituitary) were fixed in 10 per cent formol-saline solution. The removal of the pituitary gland was undertaken with particular care. All the remaining part of the skull, except the base, was removed, and the head was then positioned nose down under a dissecting microscope. With a very gentle retraction of the brain from behind, the cranial nerves were cut from the twelfth forward until the caudal end of the wax plate was seen. The position of this plate and the effectiveness of the stalk section were now carefully studied. In the goats which survived for the longer periods, adhesions were present, binding the *tuber cinereum* to the dorsal surface of the plate, and these adhesions had to be gently divided before the front part of the plate could be seen. The internal carotid arteries and optic nerves were cut and the brain was removed and fixed whole in 10 per cent formol-saline solution. The position of the head was then changed, and under the dissecting microscope a further close examination was made of the dorsal aspect of the plate covering the pituitary fossa. By

3 or 4 weeks after operation, this plate was encapsulated by well vascularized fibrous tissue. On removal of the plate, the distal end of the cut pituitary stalk could be seen in the opening of the *diaphragma sellae*. The pituitary gland was then dissected out, weighed and fixed in formol mercuric chloride (this was found to be a better fixative for the goat's pituitary than formol saline). After fixation for 18 to 24 hours, the gland was cut coronally (except one specimen which was cut sagittally) into a series of blocks which were then numbered, dehydrated and embedded in paraffin. Sections were cut at a thickness of 7 μ and stained with Ehrlich's acid hematoxylin and eosin, Weigert's iron hematoxylin and van Gieson's solution, and Masson's trichrome method. The periodic-acid Schiff, Heidenhain's azan, and Holmes' silver-on-the-slide methods of staining were also used.

Similar paraffin sections were made of the other organs, and in addition, frozen sections of various tissues were stained for fat.

Injection Preparations

Five normal male goats of the same breed as the operated animals were available for a study of the blood supply of the pituitary gland. In 2 of these animals Neoprene latex casts were made of the blood vessels of the whole pituitary area by injecting the latex into the common carotid arteries in the neck at a pressure of 400 mm. Hg. After fixation, a careful dissection was made of the pituitary region, and the origin and distribution of the individual vessels which supply the pituitary were studied. In the 3 remaining goats Berlin blue was injected, from a syringe with a fine hypodermic needle, into the artery of the lower infundibular stem (Text-fig. 3), in an attempt to obtain more precise information as to the distribution of this artery. The techniques used in making these preparations were essentially the same as those we have previously used in the sheep.[17]

Results

Clinical Observations

On the whole, the animals made a remarkably good recovery from the operation. They all lost weight during the first few days, and although later they started to gain weight, we have not enough data to assess the effect of stalk section on body growth. It was interesting, however, to note the difference in the final body weight of two particular goats (Table II). These animals (Nos. 10 and 12) were twins, operated upon on the same day when their weights were identical, and sacrificed 21 days later when it was found that in only one of them (No. 10) had the pituitary stalk been actually severed.

All the goats showed a definite polyuria and polydipsia in the first day or two after the operation. Four animals started having fits at 24 to 36 hours, and in consequence, 3 of them were sacrificed at an early stage (Nos. 6, 9 and 13); in the fourth (No. 7), the fits ceased after a week, and this animal was not sacrificed until a month after operation. We were interested to note that Davies and Buxton[4] reported the occurrence of fits in 2 human patients after stalk section. In 2 other animals (Nos. 5 and 8), there were short periods of great weakness during the first fortnight, and a few other goats were noticeably apathetic for short periods. Edema of the legs developed in one goat (No. 2) about the sixth day after stalk section, and this was pronounced at necropsy 5 days later.

Necropsy and Histologic Observations

Twelve of the 13 goats operated upon recovered from the operation. In 9 of these the stalk was found at necropsy to have been completely divided, with the wax plate lying in good position between its cut ends. In the remaining 3 goats there had been some doubt at the time of operation as to whether the stalk had been actually severed. At necropsy it was found that the stalk had not been cut, though it had been bent aside by the insertion of the wax plate.

Of the 9 animals in which the operation had been successful, 3 were sacrificed during the first 3 days after stalk section, while the others were allowed to survive for periods ranging from 11 to 29 days after the operation (Table I).

GROUP A. ANIMALS IN WHICH THE STALK HAD BEEN SEVERED

A shrinkage of the pituitary gland as a whole was noticeable macroscopically in all animals which survived for the longer periods. This was evident in a general way by comparison with the size and weight of the gland in goats of corresponding age and weight sacrificed in the first 3 days; and was particularly well shown by the difference in twin animals, operated upon on the same day and both sacrificed 21 days later, in one of which the stalk had not been cut (Table II; Figs. 5 and 6).

Anterior Lobe. In the goats sacrificed after stalk section at 25 hours, 52 hours, and 72 hours respectively, by far the greater part of the anterior lobe was the seat of infarction (Figs. 1 and 3), but small areas of surviving tissue were present in each case. These areas were situated along the dorsal margin of the lobe, particularly on its "shoulders," and in patches along the ventral border. There was also a very narrow rim of surviving cells around the remainder of the

periphery. The area of infarction contained the debris of degenerating cytoplasm and nuclei (Fig. 7). At 52 and 72 hours, the periphery of the infarct consisted of loosened tissue in which many cells with large pale nuclei were present. The boundary between living and necrotic tissue was fairly sharp and showed no inflammatory reaction (Fig. 7). In the surviving areas the epithelial cells were not entirely normal in appearance, the cells being somewhat shrunken and more closely

TABLE I

Periods of Survival After Operation

Goat No.	Survival time	Age at operation
Group A (stalk effectively cut and wax plate in good position)		
1	25 hrs.	8 mos.
6	52 hrs.	7½ wks.
9	72 hrs.	9½ wks.
2	11 days	8 mos.
3	13 days	8 mos.
8	19 days	9 wks.
10*	21 days	10 wks.
7	28 days	8 wks.
5	29 days	7½ wks.
Group B (stalk uncut but pushed aside by wax plate)		
4	3 days	8 mos.
13	5 days	11½ wks.
12*	21 days	10 wks.

* Twin goats, operated upon on the same day.

packed together than in normal goats. The nuclei were also more darkly stained than normal, though not actually pyknotic.

In the goats which survived for 11 to 29 days, the anterior lobe was evidently shrunken (Figs. 2 and 4). The necrotic area was becoming increasingly organized into well vascularized scar tissue (Fig. 8). Within it, many foci of calcification were present (Fig. 5), and these were surrounded by multinucleated foreign body giant cells (Fig. 27). In spite of the shrinkage of the anterior lobe as a whole, the area of living glandular tissue within it was larger absolutely than that seen at 1 to 3 days, the difference being particularly noticeable in the dorsal part of the lobe (Figs. 1 to 5). The anterior pituitary cells in this viable tissue had the same slightly abnormal appearance found in the first 3 days, being shrunken, closely packed together, and with rather darkly staining nuclei. Although there was much fibrosis in the region

of infarction, there was no obvious increase in fibrous tissue in the viable areas. Many of the elements bordering the infarct were clearly epithelial cells (Fig. 8), but among them were numbers of cells of histiocyte type. Intranuclear inclusion bodies were frequently seen among the living cells near the border of the infarct.

In view of the greater amount of viable glandular tissue in goats which survived for the longer periods, we made a special search for evidence of mitotic activity. Unfortunately no information on this point could be obtained from examination of the first 4 goats operated upon, as the staining of the pituitary sections, due to fixation in formol-saline solution, proved to be unsatisfactory for this purpose. Even in the other goats, with suitably stained sections, great care had to be taken in identifying mitotic figures. The debris of disintegrating nuclei may sometimes be mistaken for mitoses. Numerous unmistakable mitotic figures, however, were found in the 2 goats which survived for 52 hours and 72 hours after stalk section (Figs. 9 to 12). They were situated within the surviving tissue, near the border of the infarct. We also found mitoses in another goat, 5 days after an operation in which we had failed to cut the stalk, but by displacing the stalk with the wax plate, we had caused a unilateral infarct in the anterior lobe. In this animal the mitoses (Fig. 13) were situated among the surviving epithelial cells, slightly further from the edge of the infarct than in the other 2 goats. Mitotic figures were not found in the anterior lobe of a normal goat of similar age, and only rarely in the living anterior lobe tissue of the operated goats which survived for the longer periods (Fig. 14).

Pars Intermedia. In the animals sacrificed during the first 3 days after stalk section, the *pars intermedia* was definitely abnormal in appearance. The cells were more closely packed together than in normal goats, and the nuclei were stained more darkly. A similar picture was seen at 11 and 13 days after stalk section, but thereafter, although at the rostral end of the *pars intermedia* the cells were still unduly crowded together, at the caudal end the cells were well spread apart and closely resembled those seen in normal goats. In these longer surviving animals, particularly those which lived for 3 weeks or more, the *pars intermedia* was clearly hypertrophied (Figs. 21 and 22), especially at its caudal end. Mitotic figures were not found until 21 days after stalk section, but at this and later stages they were very numerous (Figs. 15 to 20).

Neural Tissue. In every animal all the neural tissue distal to the level at which the stalk had been cut showed evidence of degeneration.

Up to 3 weeks after the operation many "retraction balls" were seen in the most rostral part of the lower infundibular stem (the narrow strip of neural tissue, on the dorsal aspect of the gland, which connects the neural tissue of the stalk with the posterior or neural lobe; Text-figure 3). In sections stained with hematoxylin and van Gieson's mixture these "retraction balls" appeared as round, structureless globules, lying in a clear space (Fig. 25). When stained by a silver method they were found to be argentophilic, and occasionally they could be seen to be in continuity with individual nerve fibers (Fig. 28). The "retraction balls" were found lying in groups enclosed by thin bands of fibrous tissue (the normal fibrous trabeculae found at this level of the lower infundibular stem), and it was characteristic that the compartments containing "retraction balls" were singularly devoid of cells. "Retraction balls" were not found more caudally in the lower infundibular stem, nor were they seen in the goats which survived for 4 weeks.

In the first 3 days after stalk section there was an increased density in the interstitial substance both of the lower infundibular stem (Fig. 25) and of the neural lobe. The eosinophilic material which caused this increased density was often patchy in distribution. There was an apparent increase in the number of nuclei, which stained rather darkly and tended to be angular in shape.

In the goats which survived for 11 days or longer there was a progressive shrinkage both of the lower infundibular stem and of the neural lobe, with marked fibrosis (Figs. 21 and 22). Concurrently with the shrinkage, which was particularly marked in the neural lobe and the caudal part of the lower infundibular stem, the degree of cellularity and vascularity was apparently greatly increased. Large cells with big pale nuclei and a cytoplasm which was difficult to stain (Fig. 29) tended to be congregated in a somewhat less fibrotic area in the center of the neural lobe (Fig. 24). In normal goats these cells of uncertain identity are more uniformly distributed in the neural lobe.

Well impregnated silver preparations showed that 3 weeks after pituitary stalk section, the vast majority of the nerve fibers in the neural lobe had disappeared. However, a few fairly normal looking nerve fibers were still present in this tissue.

GROUP B. ANIMALS IN WHICH THE STALK HAD NOT BEEN SEVERED BUT HAD BEEN PUSHED ASIDE BY THE WAX PLATE

In 3 goats, sacrificed at 3, 5 and 21 days respectively, the stalk was found at necropsy not to have been cut but had merely been pushed aside by the insertion of the wax plate (Table I).

Anterior Lobe. In each animal there was an infarct on the left side of the anterior lobe, i.e., the side from which the plate had been pushed against the stalk (Fig. 6). In no case did the infarct extend to the midline, but the left half of the anterior lobe was shrunken. Moreover, although on the right side of this lobe the epithelial cells showed no obvious abnormality, on the left side (where the infarct was) the living cells had the same somewhat abnormal appearance as that found in the viable anterior lobe cells of the goats whose stalks had been actually severed. That is to say, the cells were rather closely packed together, with darkly staining nuclei and decreased cytoplasm. As already mentioned, mitotic figures were found among the viable anterior lobe cells near the border of the infarct in the goat which was sacrificed 5 days after operation.

Pars Intermedia. At 3 and 5 days there was no obvious abnormality on either side. At 21 days, however, the *pars intermedia* was clearly and symmetrically hypertrophied (Fig. 23) and contained many mitotic figures, thus showing the same features as those seen at 3 and 4 weeks after actual stalk section.

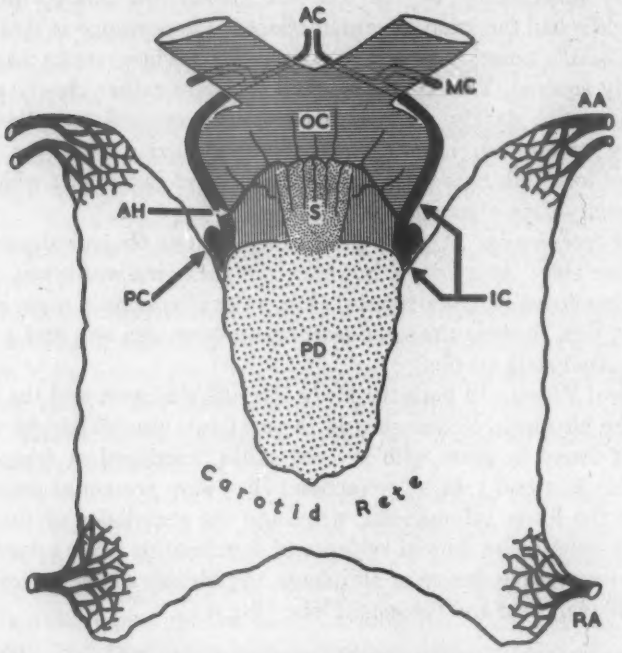
Neural Tissue. In both the lower infundibular stem and the neural lobe the histologic picture seen in these 3 goats was strikingly similar to that found in goats with severed stalks, sacrificed at comparable periods. At 3 and 5 days "retraction balls" were present at the rostral end of the lower infundibular stem, and the remainder of this stem and the neural lobe showed evidence of degeneration. At 21 days there was a remarkable degree of shrinkage and fibrosis of the entire lower infundibular stem and the neural lobe (Fig. 23).

THE BLOOD SUPPLY OF THE GOAT'S PITUITARY GLAND AND ITS BEARING ON THE RESULTS OF STALK SECTION

Most of the main features of the goat's pituitary blood supply which are relevant to the present study are shown in Text-figures 1 to 3. The vascular arrangements are very similar to those found in the sheep.[17] As in the sheep, the carotid rete[18] is a conspicuous structure in the pituitary region (Text-fig. 1). This compact mass of plexiform arteries almost completely fills the cavernous sinus on each side, and is continuous across the midline through a substantial junctional portion of the rete which lies at the back of the pituitary fossa (Text-fig. 1). The rete is thus closely apposed to the lateral borders of the pituitary gland and also surrounds its caudal end, particularly on the dorsal aspect (Text-fig. 3). On each side, close to the lateral border of the pituitary, the rete gives origin to the ipsilateral internal carotid artery.

The anterior hypophysial arteries, springing from the internal carotid

arteries on either side shortly after the posterior communicating arteries have been given off, form a ring around the upper (rostral) part of the pituitary stalk. From this ring, numerous branches run for a variable distance down the stalk to supply a first capillary bed within it (Text-figs. 1 and 3).

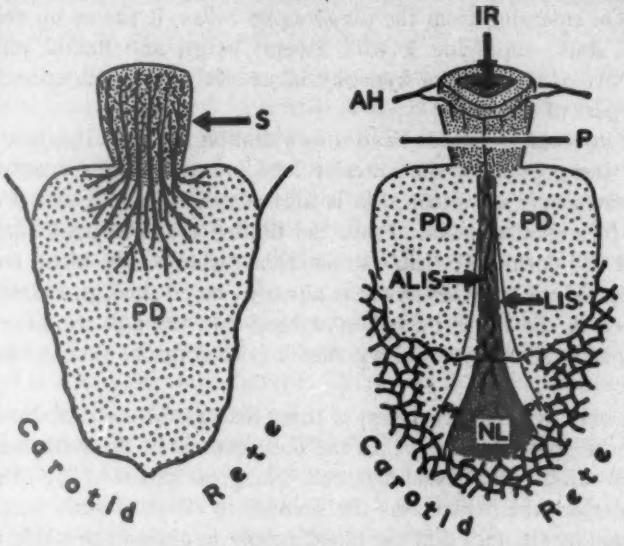


Text-figure 1. Diagram of the pituitary region of the goat, as seen from the ventral aspect, to show the general arterial topography. The carotid rete[18] surrounds the pituitary gland on its lateral and caudal aspects, and on each side the rete gives rise to the ipsilateral internal carotid artery (IC) though the origin of this artery is not seen here. Note the anterior hypophysial arteries (AH) supplying the pituitary stalk (S) from its upper (rostral) end. It is from this source that almost the whole of the anterior lobe, or *pars distalis* (PD), ultimately receives its blood supply (Text-fig. 2).

AA, arteria anastomotica
AC, anterior cerebral arteries
MC, middle cerebral artery
OC, optic chiasma
PC, posterior cerebral artery
RA, ramus anastomoticus

From this first capillary bed in the stalk the blood is drained by the hypophysial portal vessels (Text-fig. 2). These are channels of large caliber, which run down the stalk on all sides and carry the blood to a second capillary bed in the anterior lobe (*pars distalis*). These portal vessels are the sole source from which almost the whole of the anterior lobe receives its blood supply.

The posterior hypophysial arteries: As in the sheep, owing to the existence of the carotid rete, there is no single artery corresponding to the inferior hypophysial artery in man. However, the junctional portion of the rete, which surrounds the caudal end of the pituitary, fulfills the same function as the human inferior hypophysial arteries,



Text-figure 2. Diagram of goat's pituitary gland, as seen from the ventral aspect, showing the portal venous blood supply to the anterior lobe. The capillary bed in the pituitary stalk (S), supplied by the anterior hypophysial arteries (Text-fig. 1), is here seen draining into hypophysial portal vessels which run down the stalk to supply the anterior lobe (PD). These portal vessels are present on all aspects of the stalk, but for the sake of clarity they have been omitted in the dorsal view (Text-fig. 3), where the level of the stalk section is shown.

Text-figure 3. Diagram showing the arteries on the dorsal aspect of the goat's pituitary gland, and also the level at which the stalk was severed and a wax plate inserted between the cut ends (P). Note the artery of the lower infundibular stem (ALIS), springing from the junctional portion of the carotid rete (here incompletely shown, to expose the posterior, or neural lobe (NL). This artery supplies the lower infundibular stem (LIS) and the *pars intermedia* (PI). It also supplies, either directly or indirectly, the borders of the anterior lobe (PD) adjacent to the *pars intermedia* on the dorsal surface, which in coronal sections represent the "shoulders" of the gland (Figs. 1 to 6). Section of the stalk at P does not cut off the blood supply delivered by ALIS, and hence the territories supplied by this artery do not undergo infarction, as does the bulk of the anterior lobe. The arterial supply to the neural lobe (NL) from the junctional portion of the rete, which is also not disturbed by the operation, is not shown here.

AH, anterior hypophysial artery IR, infundibular recess

giving off arteries (one major and several small) to the posterior, or neural, lobe, and also giving origin to the artery of the lower infundibular stem.

The artery of the lower infundibular stem (Text-fig. 3) springs from the junctional portion of the rete on the dorsal surface of the pituitary, and runs forward in the midline in the dura mater forming the *diaphragma sellae*. In its course it gives off numerous branches to the lower infundibular stem and also to the *pars intermedia* on either side. On emerging from the *diaphragma sellae*, it passes up the back of the stalk, supplying it with several twigs, and finally joins the dorsal arc of the anterior hypophysial arterial ring which encircles the upper part of the stalk.

Our injected specimens have shown that on the dorsal surface of the gland, the portion of the anterior lobe immediately adjacent to the *pars intermedia* on either side is also supplied by the artery of the lower infundibular stem. From the limited material available, however, it has not been possible to determine whether the blood reaching this part of the anterior lobe has already passed through a first capillary bed in the lower infundibular stem and through the *pars intermedia*, as in the sheep, and whether it is thus portal venous blood, or not.

It is only by an appreciation of these features of the vascular anatomy of the goat's pituitary that the observations in the stalk-sectioned animals can be properly understood. The great extent of the infarct in the anterior lobe, which was the immediate effect of stalk section, is explained by the fact that the blood supply to almost the whole of this lobe (i.e., from the hypophysial portal vessels in the pituitary stalk) had been cut off by section of the stalk (Text-figs. 1 and 2). The small areas of the anterior lobe which regularly survive on the dorsal margins, and particularly on the "shoulders" (Figs. 1 to 5), owed their preservation to the fact that these areas are supplied with blood from the artery of the lower infundibular stem, an artery which was not damaged by the operation of stalk section except at its distal (rostral) end. Its origin and the greater part of its length were left undisturbed (Text-fig. 3). The survival of a narrow peripheral rim of cells on the lateral and ventral surfaces of the anterior lobe is probably to be explained by diffusion of oxygen from the overlying highly vascular dura mater.

Degeneration of the neural elements in the lower infundibular stem and the neural lobe was the natural consequence of stalk section, since the nerve tracts connecting these parts of the pituitary gland with the hypothalamus were severed in the stalk. These territories, however, did not undergo infarction, because they were not deprived of their blood supply.

The *pars intermedia* also derives its blood supply from the intact portion of the artery of the lower infundibular stem, and consequently the cells here did not die, although their somewhat abnormal appearance in the early stages after operation suggests that possibly this artery may have been temporarily affected by spasm.

THE EFFECTS OF PITUITARY STALK SECTION ON OTHER ORGANS

Only a preliminary examination has as yet been made of the other tissues taken at necropsy. A special study is being made of the hypothalamus, but this investigation is still at an early stage. Striking changes have been found in the other endocrine organs. The thyroid and adrenal glands of the goats which survived for more than the first few days after stalk section were atrophic. Histologically, the epithelium of the thyroid was greatly flattened. The adrenal cortex was decreased in width, and the cells in the *zona fasciculata* had lost their normal linear arrangement and were irregularly disposed and closely packed together. The nuclei of these cells were clearly shrunken and stained in unusually dark fashion. There was a marked loss of stainable lipid in the adrenal cortex.

That section of the pituitary stalk had a widespread effect throughout the body was shown by the difference found at necropsy in the body and organ weights of goats 10 and 12 (Table II). As indicated previously, these animals were twins and were operated upon on the same day when their body weights were identical. Twenty-one days later they were sacrificed, and it was found that in one, goat 12, we had failed to cut the pituitary stalk and had merely pushed it aside by the insertion of the wax plate. In goat 10, on the other hand, the stalk had been successfully severed. As shown in Table II, all the organs of this animal were very noticeably smaller than those of its twin, goat 12. It should be noted, however, that even goat 12, though providing a useful basis for comparison, was not a normal goat. Although in this animal the pituitary stalk had not been actually cut at operation, the pressure exerted on the stalk by the wax plate had caused a small infarct in the anterior lobe (Fig. 6). Moreover, the nerve tracts within the stalk had in fact been sheared, as demonstrated by the histologic changes seen in the lower infundibular stem (Fig. 23) and the neural lobe. Thus part of the primary capillary bed within the stalk (i.e., the part below the level of the wax plate) lay in a denervated area, and consequently the cells of the anterior lobe supplied by portal vessels from this denervated area were deprived of direct hypothalamic control.

Discussion

This study has shown that section of the pituitary stalk in the goat produces a massive infarction of the anterior lobe of the pituitary gland, just as it does in the sheep.[15,16] This infarction is due to the fact that the only blood supply to virtually the entire anterior lobe is that which flows down the hypophysial portal vessels in the pituitary stalk, and this blood supply is of course cut off when the stalk is severed.

The blood supply to the anterior lobe of the goat's pituitary is strictly comparable to that of the human anterior lobe.[19,20] That is to

Table II

Weights of Organs of Twin Goats, Operated Upon on the Same Day and Sacrificed 21 Days Later

	Goat 10 stalk cut	Goat 12 stalk uncut but damaged
Body weight at operation	11.4 kg.	11.4 kg.
Body weight at necropsy	11.5 kg.	14.1 kg.
Brain	75 gm.	84 gm.
Heart	50 gm.	72 gm.
Liver	303 gm.	450 gm.
Kidney, R.	31 gm.	42 gm.
Kidney, L.	33 gm.	44 gm.
Spleen	19 gm.	27 gm.
Pancreas	13 gm.	24 gm.
Thymus	16 gm.	30 gm.
Testis, R.	4.5 gm.	10 gm.
Testis, L.	4 gm.	9 gm.
Thyroid, R.	0.42 gm.	0.58 gm.
Thyroid, L.	0.39 gm.	0.62 gm.
Adrenal, R.	0.35 gm.	0.62 gm.
Adrenal, L.	0.40 gm.	0.71 gm.
Pituitary	0.21 gm.*	0.29 gm.

* With much dura still attached.

say, the sinusoids of the anterior lobe constitute a second capillary bed which receives only portal venous blood. The first capillary bed of this vascular system, which is supplied by the anterior hypophysial arteries, is situated in the pituitary stalk. Connecting the two capillary beds are the hypophysial portal vessels, which carry the blood from the stalk to the anterior lobe.

Histologically, the appearance of the acute lesion induced in the goat's anterior pituitary by stalk section is essentially the same as that

seen in the anterior lobe of rats and sheep after interruption of the portal blood supply from the pituitary stalk.[14-16] The lesion also closely resembles that described by Russell[21] in a case of accidental pituitary stalk section in man. Further, the pathologic changes seen in the anterior lobe of the human pituitary in acute postpartum anterior pituitary necrosis[22] are very comparable with those seen in the anterior lobe after stalk section in our goats. Thus the results of the present experiments, particularly when added to the observations in rats and sheep,[14-16] lend strong support to our view[20] that infarction of the anterior lobe in human postpartum pituitary necrosis is the result of a severe impairment of the circulation in the pituitary stalk. The impairment could be due to an obstruction to the blood flow in one of 3 sites; namely, in the anterior hypophysial arteries, in the peculiar vessels of the first capillary bed (Figs. 6 to 9, and 16 in Xuereb, Prichard and Daniel[20]) or in the hypophysial portal vessels themselves.

In our previous experiments on rats and sheep only the very early effects of operative interference with the stalk had been investigated. Even in the present study on goats, no animal was allowed to survive for more than 4 weeks, but in spite of this relatively short time there was a suggestion that some regeneration of anterior lobe tissue had occurred after the first few days. Since the necropsy examinations had shown that the wax plate had formed an effective barrier between the cut ends of the stalk, there was no question of regeneration of the hypophysial portal vessels[23-25] having occurred. Nevertheless, in spite of the severe shrinkage of the anterior lobe, the amount of the viable portions of this lobe was in all cases noticeably greater in the longer surviving animals than that found in the goats which were sacrificed in the first few days after stalk section. Mitotic figures were frequently seen near the borders of the infarcts in goats sacrificed at 2, 3 and 5 days after operation. They were, however, only rarely found in animals sacrificed at later stages, a finding which is hard to explain if any appreciable regeneration was still taking place. That mitotic division of anterior lobe cells does occur has been shown by numerous workers; e.g., mitoses have been seen in the rat,[26-32] in the mouse,[33,34] and in the monkey.[35] In general, however, these observations have been made on females and have been related either to certain stages of the estrus cycle or to the administration of estrogenic substances. We have ourselves seen intense mitotic activity in the anterior lobe of young ovariectomized female rats which had been given estrogen.[36] Moreover, we were particularly interested to find mitotic figures in a surviving fragment of anterior lobe tissue both in a woman after "total"

hypophysectomy and in a case of postpartum anterior pituitary necrosis.[37] We do not know whether the mitotic figures in the stalk-sectioned goats were specifically associated with regeneration or whether they were evidence of a normal process of growth in a young animal, but it was of interest that the mitoses were in all cases near the border of the infarct. More experiments are needed before any firm conclusion can be reached as to whether or not the surviving anterior lobe cells regenerate and grow into the area of infarction.

Turning now to the possibility of using pituitary stalk section as an alternative to hypophysectomy in man for the control of certain types of carcinoma, it should be stressed that section of the pituitary stalk will not cause necrosis of the entire anterior lobe. With this operation, the blood supply to a small part of the anterior lobe, i.e., the supply transmitted through the short portal vessels from the lower infundibular stem, is left intact (Fig. 16 in Xuereb, Prichard and Daniel[20]). Moreover, all the reports of extensive infarction of the anterior pituitary in man mention a narrow layer of surviving anterior lobe cells around the periphery of the lobe, similar to that seen in our experimental animals. However, even in "total" hypophysectomy, fragments of the anterior lobe are usually unavoidably left behind in the *sella turcica*.

If the presence of surviving remnants of anterior lobe tissue is found to defeat the object for which the operation is performed, the question as to whether regeneration does or does not occur becomes all the more important. It is equally important to know whether the surviving anterior lobe tissue has any functional activity relevant to the carcinomatous process when it is separated from the hypothalamus, a center which is now regarded as intimately associated with the regulation of anterior lobe activity.[38] It seems probable that the anterior lobe cells, deprived of the blood which they normally receive from the hypophysial portal vessels in the stalk, have their secretory activity so severely limited that they become unimportant endocrinologically. This possibility is supported by our observation in the goat that even the surviving anterior lobe cells were not entirely normal in appearance, a finding which has also been recorded in stalk-sectioned rabbits by several workers.[5-8,39] The somewhat abnormal appearance of the viable cells after stalk section is probably due to the fact that although these cells have had an adequate blood supply to preserve them, the blood supply has not been from the right source and therefore has not been of the right nature to maintain them as fully functioning anterior lobe cells. More specifically, the blood which the living cells have received has not come from the pituitary stalk, and

thus has not carried with it the substances derived from this vital region. The fact that stalk section diminishes and even abolishes certain types of anterior lobe activity has been shown by the work of Harris and his colleagues,[9-11,38,40] whose findings are all the more interesting since they were obtained in rabbits, in which stalk section rarely causes infarction in the anterior lobe.

If it can be shown that the persisting remnants of anterior lobe tissue, having been deprived of their hypophysial portal blood supply, have lost those functional activities which include stimulation of the carcinomatous process, then section of the pituitary stalk could supersede the technically more difficult operation of hypophysectomy.

The shrinkage of all the pituitary neural tissue distal to the level of the stalk section is only to be expected after the nerve tracts in the stalk have been severed. Atrophy of the neural lobe after stalk section has been observed in the rat,[41-43] in the rabbit,[5,9,39] and in the monkey,[44] but mention is rarely made of fibrosis, which in our goats was such a striking accompaniment of the shrinkage in both the lower infundibular stem and the neural lobe.

The great hypertrophy of the *pars intermedia* in our longer surviving goats was another interesting finding. Enlargement of the *pars intermedia* after stalk section has been reported in rats by Uotila[41] and Barrnett and Greep,[42] and in rabbits by Brooks.[5] On the other hand, Campbell and Harris[9] thought that there was no increase in the volume of this tissue in their stalk-sectioned rabbits. The explanation of the hypertrophy of the *pars intermedia* in our goats remains to be determined, but the observations in one goat (No. 12) suggest that it was related to the degeneration of the neural tissue in the lower infundibular stem and the neural lobe, rather than to the presence of a large infarct in the anterior lobe. For in this animal, in which the *pars intermedia* was greatly hypertrophied, there was only a small, unilateral infarct in the anterior lobe (owing to our failure to cut the stalk), but the neural tissue of the lower infundibular stem and the neural lobe had completely degenerated (Figs. 6 and 23).

Summary

The pituitary stalk was cut in 9 goats, and a wax plate was inserted between the severed ends. This caused a massive infarction of the anterior pituitary lobe (*pars distalis*) though certain areas of this lobe regularly survived. The areas of viable anterior lobe cells were larger in the goats which were allowed to survive for from 1½ to 4 weeks than in those sacrificed in the first 3 days after stalk section. This observation, and the presence of mitotic figures among the surviving

cells near the borders of the infarct, suggest that some regeneration of anterior lobe tissue had occurred. More experiments are needed, however, before this point can be definitely established. The living anterior lobe cells showed a slightly abnormal appearance in both the early and the later stages after stalk section.

The neural portions of the pituitary gland showed evidence of degeneration. "Retraction balls" were found in the lower infundibular stem near the level of the stalk section, and both the lower infundibular stem and the posterior, or neural, lobe showed a progressive shrinkage and fibrosis.

The *pars intermedia* became hypertrophied, and many of its cells were undergoing mitosis.

Marked changes were seen in other tissues of the body, particularly in the other endocrine organs.

In 3 goats the pituitary stalk was not actually cut at operation, but was bent aside by the insertion of the wax plate. In these animals there was only a small, unilateral infarct in the anterior lobe, but histologic changes in the lower infundibular stem, the neural lobe and in the *pars intermedia* were similar to those seen after section of the pituitary stalk.

The changes observed in these experiments are explained in the light of the vascular arrangements of the pituitary gland of the goat, which are very comparable with those of the human pituitary.

The results are briefly discussed in relation to human postpartum necrosis of the anterior pituitary, and to the possibility that in man, section of the pituitary stalk might effectively replace hypophysectomy in the treatment of certain types of carcinoma.

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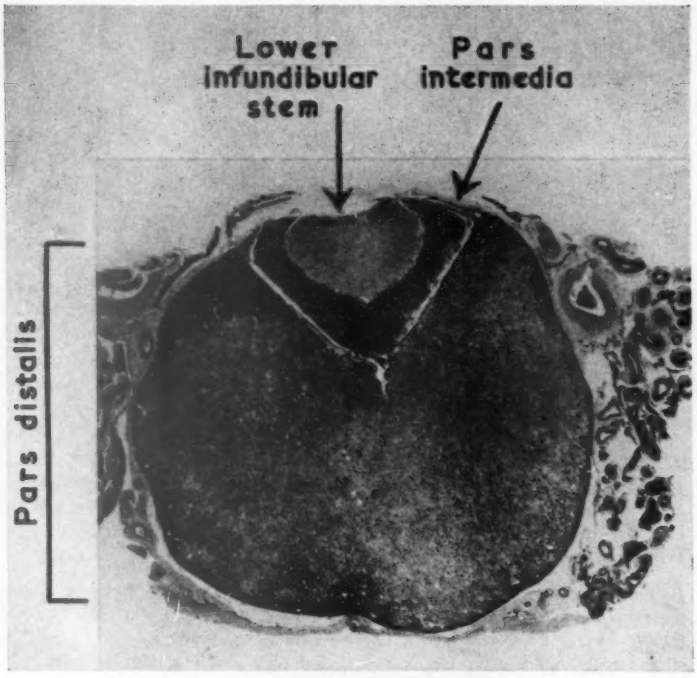
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIGS. 1 to 6. Coronal sections through the pituitary glands of 6 goats, showing the extent of the infarct produced in the anterior lobe. These are shown in 3 pairs for comparison, each pair of sections being taken from goats of a similar age on the day of operation and illustrating a comparable level in the gland. In each photograph (and as indicated in Fig. 1) the anterior lobe, or *pars distalis*, is represented by all that part of the gland seen below and lateral to the V-shaped cleft (residual lumen of Rathke's pouch), the *pars intermedia* by the band of tissue bordering the cleft above and medially, and the lower infundibular stem by the tissue seen at the top center of the gland, largely or even completely surrounded by the *pars intermedia* (Text-fig. 3; Figs. 21 to 23). In each case, the infarct in the anterior lobe is seen as a pale area, and the regions of living anterior lobe cells are dark.

FIG. 1. Goat No. 1, 25 hours after pituitary stalk section. Almost the entire anterior lobe has undergone infarction (pale). The surviving anterior lobe cells (dark) are confined to a small patch on each "shoulder" of the gland, and to a narrow rim around the periphery of the anterior lobe. Hematoxylin and eosin stain. × 10.5.

FIG. 2. Goat No. 2, 11 days after stalk section. Compare with Figure 1, and note the smaller size of the anterior lobe and the more extensive area of living cells within it. The lower infundibular stem is shrunken. Masson trichrome stain. × 10.5.



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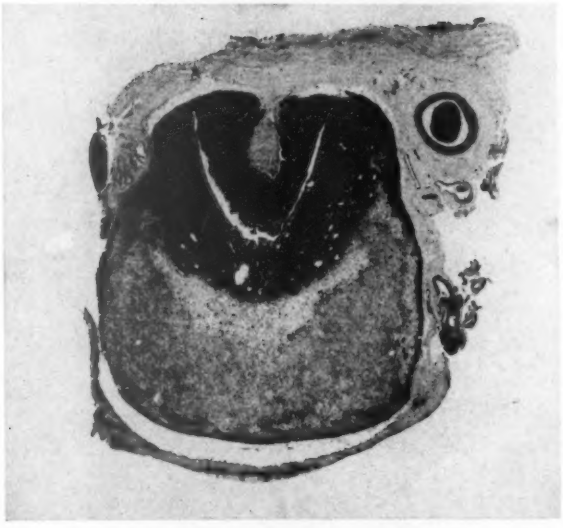

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FIG. 3. Goat No. 9, 3 days after pituitary stalk section. Only a thin layer of cells around the periphery of the anterior lobe survives, together with a small area on each "shoulder." There is infarction of all the rest of the anterior lobe. Hematoxylin and eosin stain. × 14.

FIG. 4. Goat No. 7, 28 days after stalk section. Compare with Figure 3, and note the greater extent of the living tissue in the anterior lobe. Note also the hypertrophy of the *pars intermedia* and the shrinkage of the lower infundibular stem (see also Fig. 22). Hematoxylin and eosin stain × 14.

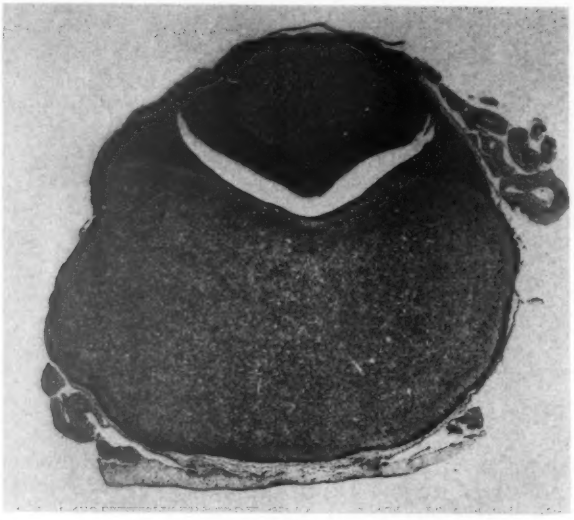

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FIG. 5. Goat No. 10, 21 days after pituitary stalk section. Compare with Figure 6 (this goat's twin, operated upon on the same day), and note the shrinkage which the anterior lobe has undergone. There is calcification in the left side of the infarct. The area of viable anterior lobe cells is, however, considerably greater than that seen in the first few days after stalk section (compare with Figs. 1 and 3). Hematoxylin and eosin stain. × 14.

FIG. 6. Goat No. 12, 21 days after an operation in which the pituitary stalk had not been severed, but had been merely bent aside by the insertion of the wax plate. A small infarct is seen on the left side of the anterior lobe, which is, moreover, somewhat shrunken on this side. The lower infundibular stem is also shrunken and fibrosed. Hematoxylin and eosin stain. × 14.

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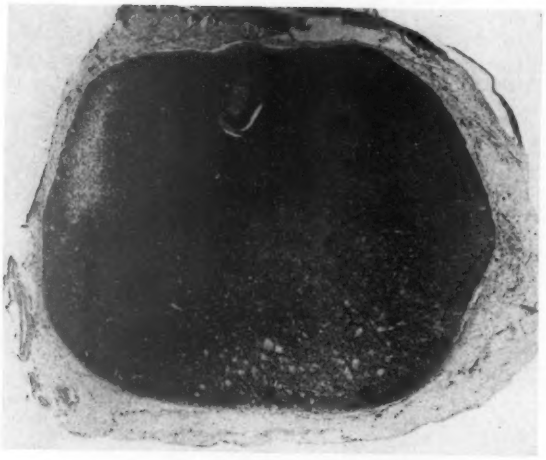

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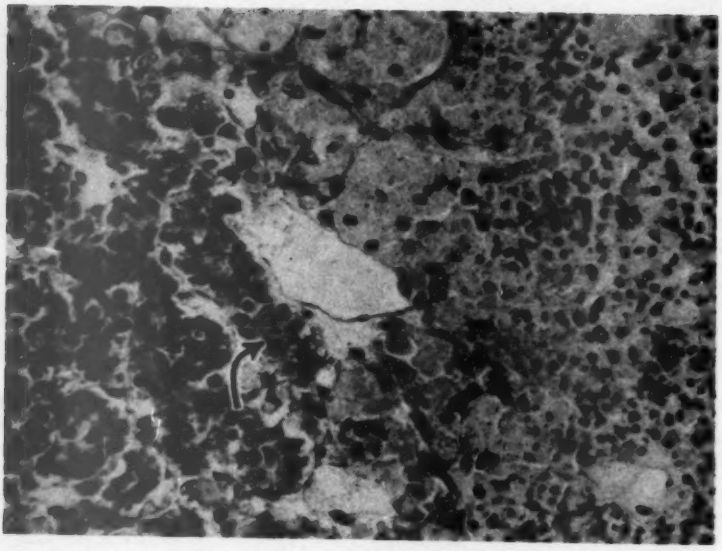
FIG. 7. Edge of infarct in anterior lobe of a goat (No. 6) sacrificed 52 hours after stalk section. The necrotic area is on the right and shows the debris of degenerating nuclei and cytoplasm. There is a band of loosened tissue, containing cells with large pale nuclei, along the border of the infarct (center). The cells on the left are part of an area of surviving anterior lobe tissue. They are somewhat shrunken and crowded together, and their nuclei have stained rather darkly but are not pyknotic. A mitotic figure can be seen (arrow) near the border of the surviving cells. Hematoxylin and eosin stain. × 174.

FIG. 8. Edge of infarct in anterior lobe of a goat (No. 7) sacrificed 4 weeks after stalk section. The area of infarction (upper right) is fibrotic and well vascularized. Living anterior lobe cells are seen (lower left) extending to the border of the infarct. Hematoxylin and eosin stain. × 174.



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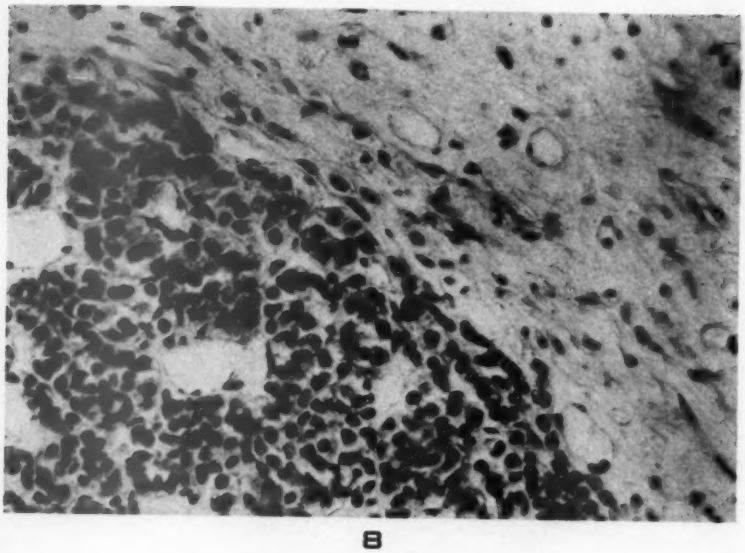

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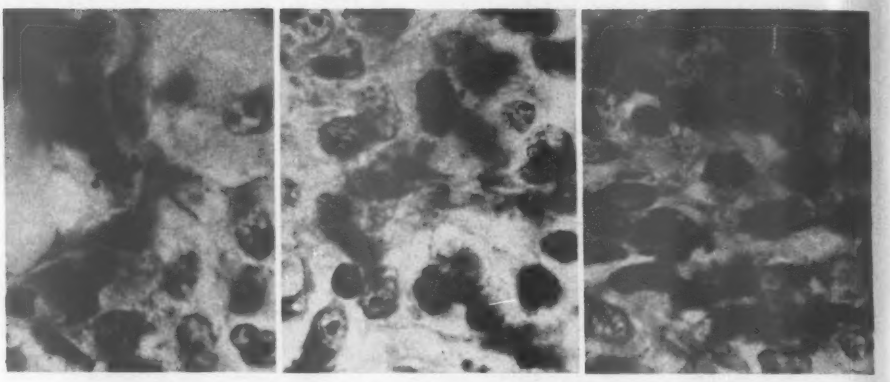
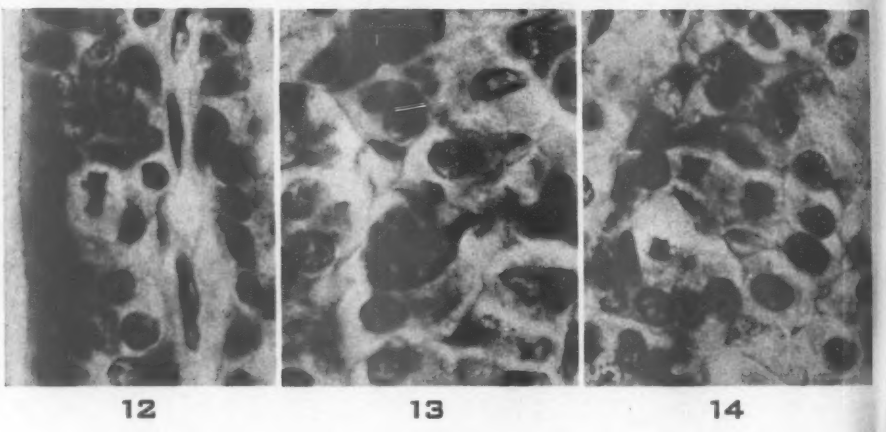
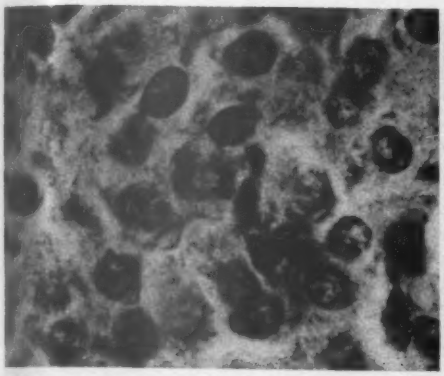




FIGS. 9 to 14. Mitotic figures found among the viable anterior lobe cells near the border of the infarct, in the first few days after operation (Figs. 9 to 13), and at 28 days after stalk section (Fig. 14). Figures 9 to 13, hematoxylin and eosin stain; Figure 14, periodic acid-Schiff stain. × 1,000.

FIGS. 15 to 20. Mitotic figures in the *pars intermedia* of a goat (No. 7) sacrificed 4 weeks after stalk section. By this stage mitoses were very numerous in this hypertrophied tissue (see Figs. 21 to 23). Hematoxylin and eosin stain. × 1,000.



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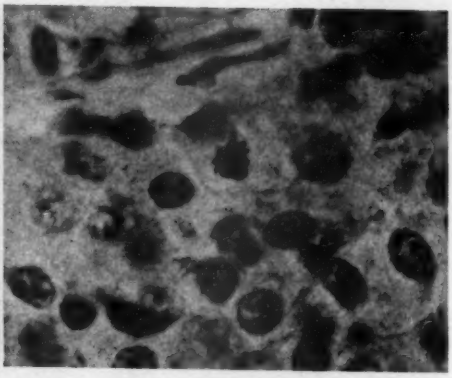

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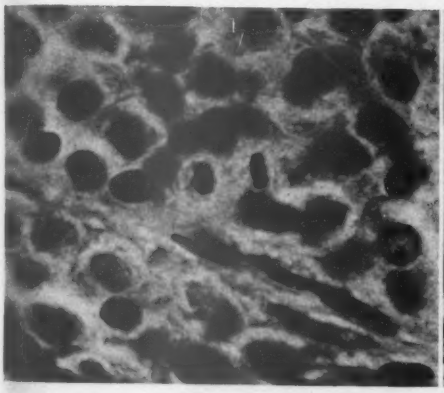

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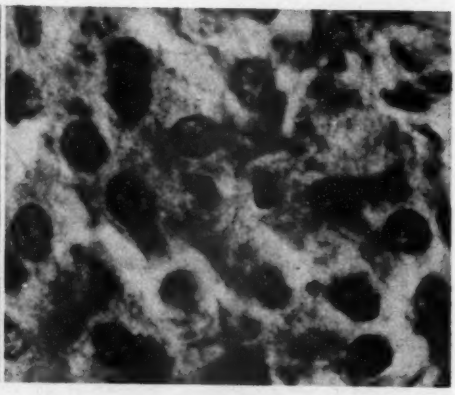

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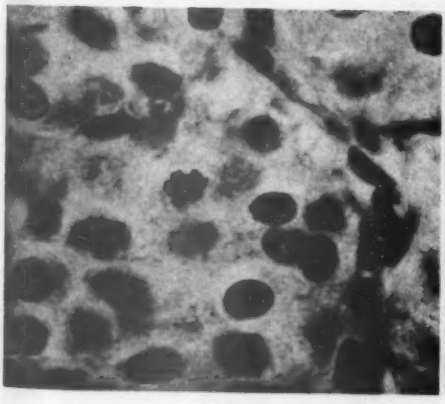

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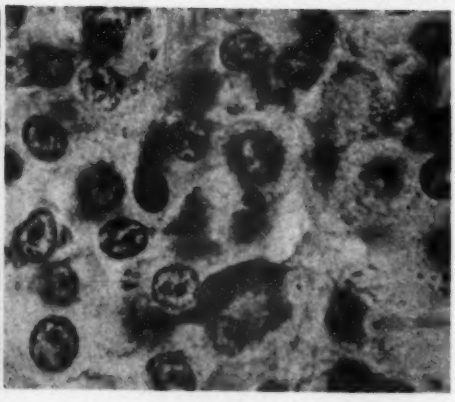

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FIGS. 21 to 23. Coronal sections cut at approximately the same levels through the pituitary glands of 3 goats, showing the marked hypertrophy of the *pars intermedia* (PI) and the shrinkage and fibrosis of the lower infundibular stem (LIS) which occurs after damage to the pituitary stalk. The hypertrophy of the *pars intermedia* is associated with intense mitotic activity in this part of the gland (see Figs. 15 to 20). PD, *par distalis* (anterior lobe), the condition of which cannot be assessed from these pictures, since the sections were stained to show particularly the fibrous tissue in the lower infundibular stem. The 3 sections were stained with van Gieson's mixture and with minimal iron hematoxylin. × 30.

FIG. 21. Goat No. 6, 2 days after stalk section.

FIG. 22. Goat No. 7, 28 days after stalk section.

Fig. 23. Goat No. 12, 21 days after the stalk had been pushed aside by the wax plate, but not severed.

FIG. 24. Central part of neural lobe of a goat (No. 7), sacrificed 4 weeks after stalk section. Note the intense fibrosis (top and bottom), surrounding a less fibrotic area where many large cells with big nuclei are congregated (see Fig. 29). Hematoxylin and eosin stain. × 216.

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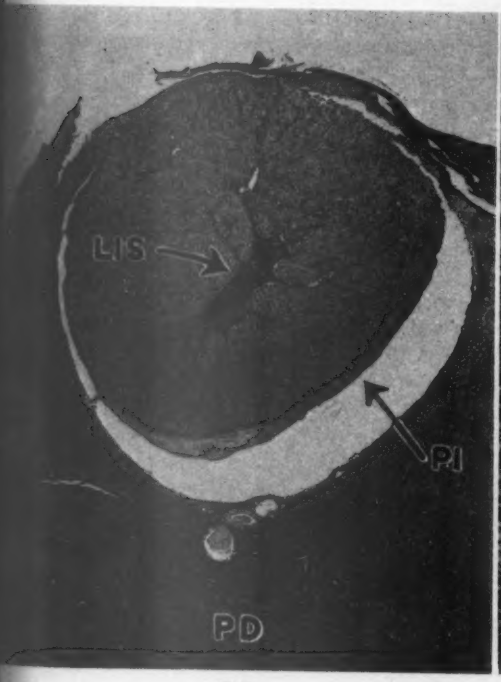

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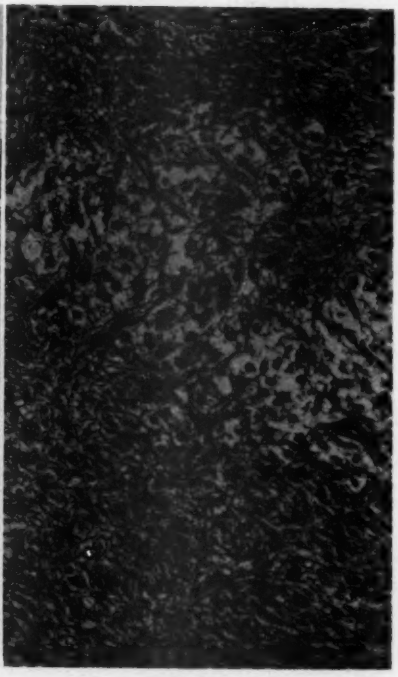

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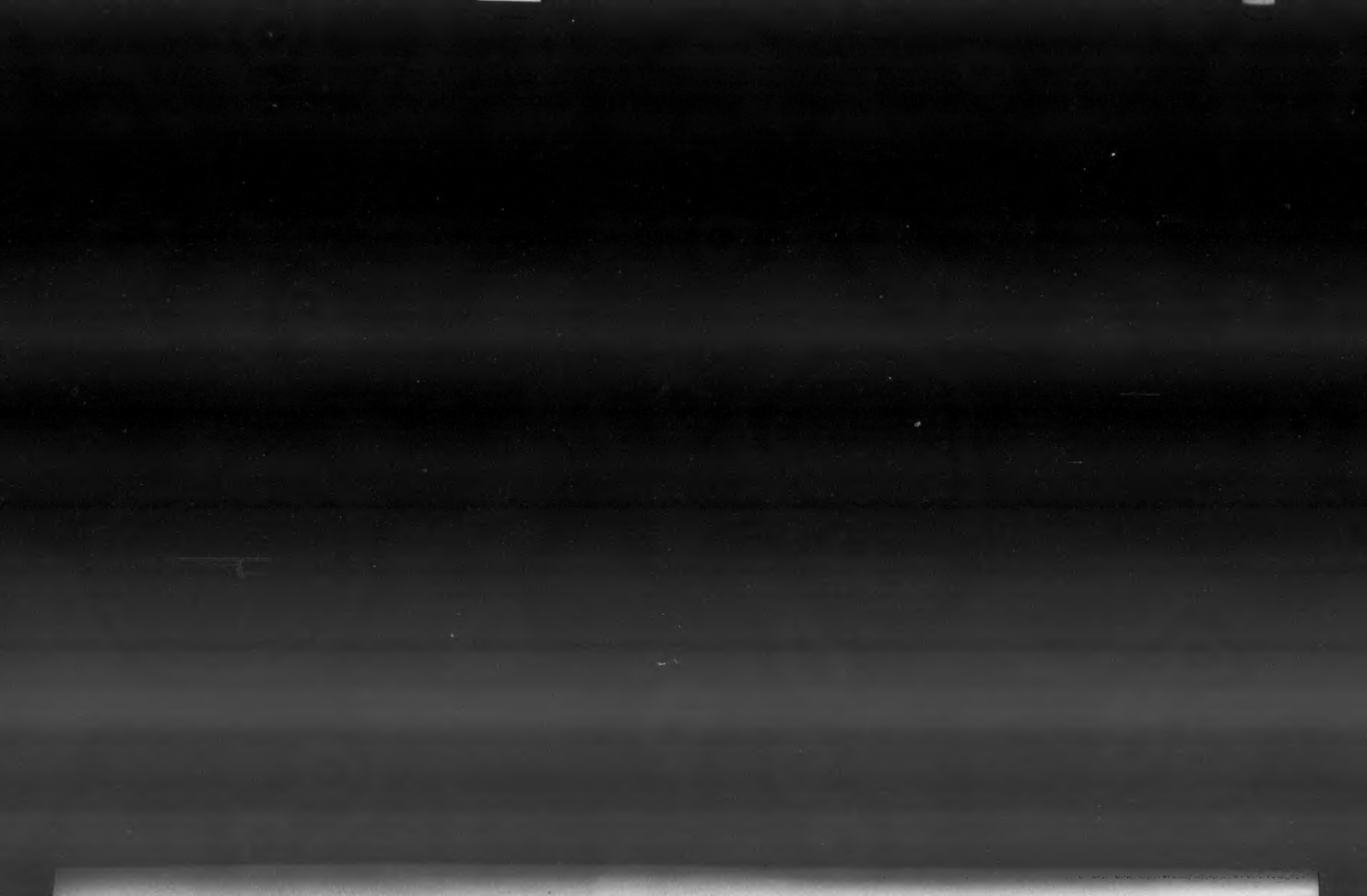
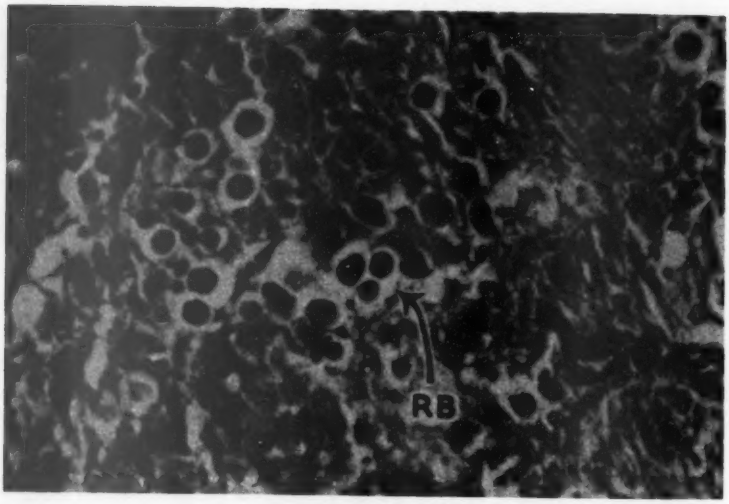
FIG. 25. Part of the lower infundibular stem, near its rostral end (i.e., near the stalk) showing a number of "retraction balls" (RB), 3 days after the stalk had been damaged but not severed (goat No. 4). These "retraction balls" (which are argentophilic; see Fig. 28) are seen here as round structureless globules surrounded by a clear space. Note also (by comparison with Fig. 26) the increased density of the interstitial substance. Iron hematoxylin and van Gieson stain. × 600.

FIG. 26. Corresponding area of the lower infundibular stem of a normal goat, for comparison with Fig. 25. Iron hematoxylin and van Gieson stain. × 600.





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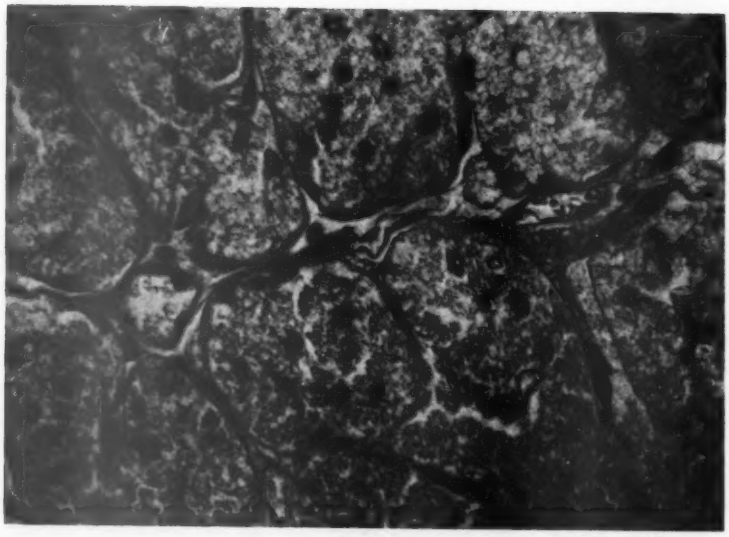

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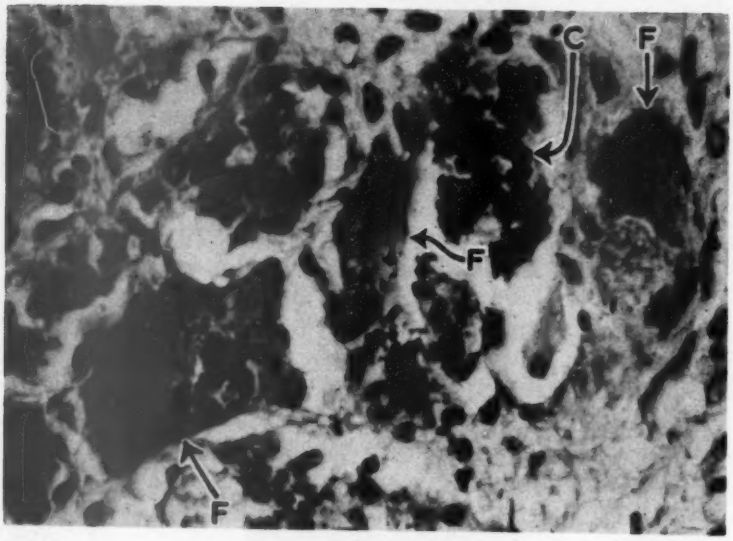
FIG. 27. Multinucleated foreign body giant cells (F) and foci of calcification (C) in the area of infarction in the anterior lobe, 29 days after stalk section (goat No. 5). Note the fragments of calcified tissue within the foreign body giant cell in the lower left corner. Hematoxylin and eosin stain. × 600.

FIG. 28. "Retraction balls" in the rostral part of the lower infundibular stem, as seen in a section stained by a silver method (see also Fig. 25). Note that two of these argentophilic "retraction balls" show individual nerve fibers actually entering or leaving them. Holmes' stain. × 1,000.

FIG. 29. One of the large cells with big pale nuclei found in the neural lobe of the goat, which at 3 to 4 weeks after stalk section tended to be congregated together in a less fibrotic area in the center of this lobe (see Fig. 24). The identity of these cells is not yet known. Hematoxylin and eosin stain. × 1,000.



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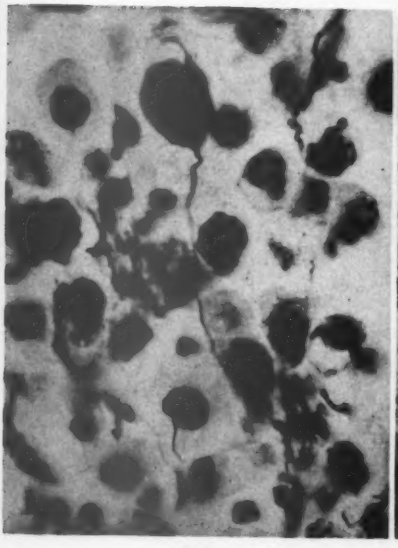

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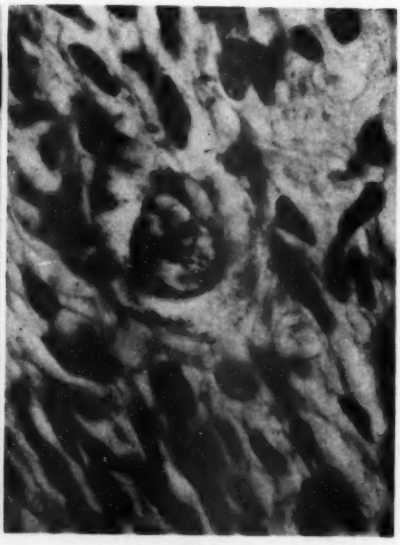

29

EXPERIMENTAL STUDIES OF THE *IN VIVO* RELATIONSHIPS OF THE PROPERDIN SYSTEM TO RESISTANCE TO INFECTION*

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The original description of the properdin system in serum[1] and the discovery that its properties are not due to specific antibodies have led to a re-evaluation of the mechanisms of host resistance.[2] In recent reviews,[2-4] the neutralizing or killing effects of properdin on a number of viruses, bacteria, and protozoa, and its relation to certain hemolytic phenomena have been reported. In addition, the depression of serum properdin levels in a number of experimental and clinical disease states, including shock,[5] viremia, bacteremia,[2] total body radiation,[2] and surgical trauma, has been described.[2]

These observations indicated the need for further study of the relationship between serum properdin levels and resistance to infection. In addition, *in vivo* studies are required to establish the extent to, or manner in which a rise or fall in the serum properdin level reflects a corresponding change in host resistance to infection. Over a period of several years, a series of animal experiments were performed for the purpose of elucidating these questions. The results are herewith reported.

Initially, it was shown that zymosan[1,2] combined with properdin *in vitro* in the presence of Mg^{++} and certain serum factors resembling components of complement. Shortly after this observation, the intravenous injection of zymosan into laboratory animals was shown to alter serum properdin levels.[6] Rowley[7,8] showed that the cell wall residues (polysaccharides) of *Escherichia coli*, as well as zymosan, altered the resistance of mice to *E. coli* infections. Later, polysaccharide complexes of other microbial and mammalian origins were also shown to alter properdin levels and resistance.[9-11]

Zymosan,† the polysaccharide used in the studies reported here, has many features favorable for animal studies. One is that zymosan is used in the purification and assay of properdin. Another is that the injection of zymosan elevates properdin levels for longer periods than

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† Zymosan is the name applied to the insoluble residue of ordinary baker's yeast following exhaustive autolysis, trypsin digestion and alcohol precipitation.

most other polysaccharide complexes. Furthermore, it is well tolerated by most experimental animals.

The present studies show that parenteral administration of zymosan results in alterations of the titer of serum properdin which is, in turn, reflected in alterations of resistance to infection over a wide range of time-dose relationships. Although a causal relationship between serum levels of properdin and host resistance may be inferred from these data, it is recognized that other mechanisms of resistance may well be involved. Therefore, studies of alterations in host resistance were carried out following the parenteral administration of purified properdin *per se*. Enhanced resistance to infection was demonstrated in mice whose serum properdin levels were elevated as a result of either zymosan or properdin administration.

Materials and Methods

CF-1 female mice weighing between 15 and 18 gm. were obtained in multiples of 100. These mice were observed for 7 to 14 days before being used in an experiment and were maintained at all times on feed free of antibiotic agents. Only animals showing a weight gain during the observation period were used in the experiments. All animals were housed in groups of 10 to 20 per cage in humidity- and temperature-controlled quarters. Experiments were designed so that mice received in a single shipment were used in a single experiment. Census of daily survivors and frequent individual or group weights were obtained.

Partially purified properdin, prepared by the zymosan elution procedure, was used.[1,12] This contained 1,000 to 1,500 units per mg. of nitrogen, as determined by the zymosan assay for properdin[12] and micro-Kjeldahl analyses. Properdin samples were stored at −30° C.

Intravenous injections via the tail vein were readily and rapidly accomplished following a 5 minute warming period of the whole mouse in an incubator at 50° to 55° C. Injections were made using a 24 gauge ¾ inch hypodermic needle. Doses up to 0.5 ml. were well tolerated.

Mice were anesthetized with 15 to 25 mg. per kg. of Nembutal® (veterinary) by the intraperitoneal route. Anesthetized mice were pinned to a cork board, and the thoracic cavity was entered through two oblique incisions in the rib cage, flapped at the sternal notch. After incision of the pericardium, the left side of the heart was grasped gently with blunt forceps. Simultaneously, the right side of the heart was punctured with the capillary tip of a 2.0 cc. Pasteur pipette. Blood was aspirated into the pipette and transferred to test tubes. The blood from individual pipettes was allowed to clot for 30 minutes. The clots

were then rimmed and centrifuged at low speed for 3 minutes. Serums collected from similarly treated mice were pooled and frozen at –30° C. until assayed. Pooled samples of approximately 1.5 ml. of serum were usually obtained from the blood of 5 mice.

Klebsiella pneumoniae, strain AD (obtained from Dr. J. F. Kiser), was grown on beef broth agar slants at 37° C. for 5 hours. The growth of several slants was combined and suspended in approximately 10 ml. of physiologic saline. These suspensions were centrifuged in calibrated conical centrifuge tubes for 10 minutes at 1,500 r.p.m. and the supernate discarded. The bacterial sediment was resuspended in saline and centrifuged a second time and the supernate was again discarded. The bacterial button obtained was diluted tenfold in physiologic saline and constituted the initial working suspension of organisms. Dilutions for injection were made from this suspension and were plated on culture media and counted 24 hours later. The intraperitoneal LD_{50} for mice was 30 organisms or fewer. Approximately this number of organisms was contained in 0.5 ml. of 1×10^{-8} dilution of the working suspension.

Experimental Observations

Effect of E. coli Infection on Serum Properdin Levels in Mice

Since the parenteral administration of zymosan and bacterial cell walls alters serum properdin levels of normal mice,[6] it appeared of interest to study the effect on serum properdin levels of infection with whole, live bacteria.

Four groups of 47 mice received doses of *E. coli* ranging from 3.4×10^4 to 3.4×10^9 organisms in 0.5 ml. of saline intraperitoneally. Properdin levels in pooled serum of 5 mice from each group were determined at 1, 2, 24 and 72 hours following inoculation and compared with serum levels of uninfected groups. The survival of the 27 remaining infected mice in each group and an untreated control group was followed for periods up to 14 days following inoculation.

Following the highest dose injected (3.4×10^9 organisms), the serum properdin titers of mice (Table I) fell to approximately 10 per cent of the original value. All of the 27 remaining infected mice died within 24 hours. No deaths occurred in the untreated control group. Following a tenfold decrease in dose (3.4×10^8 organisms), there was a progressive fall in serum titer to 20 per cent of the original level between 2 hours and 24 hours following inoculation. All of the 27 remaining infected mice died within 72 hours.

Following the introduction of 2 lower doses of organisms (3.5 million and 35 thousand organisms respectively), there was a transient fall of 50 and 25 per cent in serum properdin levels demonstrated 24

hours after injection, with a 15 per cent death rate in both groups. Serum properdin levels in both of these groups had returned to normal in 72 hours.

TABLE I

Properdin Levels and Survival of CF-1 Mice Following Inoculation with E. coli

Number of *E. coli* injected intraperitoneally*	Hours elapsed between inoculation and bleeding				Survivors (14 days)†
	1	2	24	72	
	Properdin level (units per ml. of serum)				
3.4×10^4	12	12	9	12	23/27
3.4×10^6	12	12	6	12	23/27
3.4×10^8	12	6	2		0/27 (3 days)
3.4×10^9	1	1	1		0/27 (1 day)

* 47 mice were inoculated at each dose.
27 mice in each group for determination of survivors.
Control serum properdin level, 12 units per ml.
† Data presented as survivors divided by total mice used in determination (S/T).

Alterations in the Serum Properdin Levels of Female Mice (CF-1) and Male Rats (Wistar Strain) Following the Administration of Zymosan

Administration of Zymosan to CF-1 Mice. The intravenous injection of zymosan (Type A)[12] into mice results in an initial fall in serum properdin level followed by a secondary rise which is dependent upon the time-dose relationship of zymosan administration.[6] As a continuation of these earlier observations, the rate, extent, and duration of

TABLE II

Serum Properdin Levels of CF-1 Mice Following Intravenous Administration of Zymosan (Time-dose Relationship)

Zymosan mg. per kg.	Serum properdin levels (units per ml.) at specified bleeding times following zymosan administration*							
	Minutes		Hours	Days				
	5	30	1 to 4	1	3	5	10	12
5.0	6	6	6	12	24	24	24	18
25.0	6	6	6	6	24	24	24	12
125.0	2	2	2	3	6	9	9	

* Pooled serum from 4 to 6 untreated mice contained 12 units per ml.

the alterations of serum properdin levels following the introduction of zymosan were investigated. Groups of 15 to 20 mice received amounts of zymosan in saline in the tail vein to yield final doses of 5, 25, or 125 mg. per kg. of body weight.

Beginning at 5 minutes and continuing at given intervals up to 12 days after the administration of zymosan, 4 to 6 mice from each group were bled and properdin levels determined on pooled serum samples.

Five minutes following the injection of zymosan (Table II), serum properdin levels were 50 per cent of the original level after a dose of either 5 or 25 mg. per kg., and 16 per cent after a dose of 125 mg. These titers persisted at their respective lowered levels up to 4 hours. Between 4 and 24 hours following the injection of zymosan, serum properdin levels returned to 100 per cent of original titers after a dose of 5 mg. per kg., 50 per cent after 25 mg., and 25 per cent after 125 mg. Seventy-two hours following the administration of zymosan, serum properdin levels were 200 per cent of the original titers after 5 and 25 mg., and 50 per cent after 125 mg. Between 3 and 10 days after the introduction of 5 and 25 mg. of zymosan, serum properdin levels were maintained at 200 per cent of the original titers, while after 125 mg., the serum titer rose only to 75 per cent of the original titer. At 12 days after the administration of 5 mg., the serum titer had fallen to 150 per cent, and after 25 mg., the serum titer was 100 per cent of the original level.

Following the intravenous injection of doses of zymosan greater than 125 mg., with the particular zymosan preparations used, acute systemic manifestations were noted. These consisted of transient episodes of respiratory distress, ruffled fur and, in many instances, bloody diarrhea and blood-tinged urine. These phenomena were observed within 5 to 30 minutes after the introduction of zymosan and cleared spontaneously by 24 hours. The intravenous injection of approximately 200 mg. of zymosan usually resulted in rapid death within 2 to 5 minutes. Necropsy examination of these mice revealed multiple acute pulmonary vascular occlusions by emboli. In sections prepared with the McManus periodic acid-Schiff (PAS) stain, the emboli consisted of clumped, pink-staining masses, probably representing aggregates of zymosan.

The serum properdin levels of mice, following the intraperitoneal injection of zymosan in saline, were also studied following single doses ranging from 5 mg. per kg. to 125 mg. Properdin levels were determined of pooled serum from groups of 5 mice bled at 2 hours, 24 hours, 4 days and 10 days following injection. Following all dosages tested, there was a prompt fall to 50 per cent (6 units per ml.) of the original level within 2 hours after introduction. Following 5 mg., the serum level rose to 200 per cent of the original value by 24 hours and was elevated to this level for a period of 10 days. Following 25 and

125 mg., a depressed serum level (50 per cent) was maintained for 24 hours and in both instances rose only to normal value between 4 and 10 days after the administration of zymosan.

Injection of Zymosan into Rats. A similar study was carried out using 175 gm. rats (Wistar strain), following intravenous injection of zymosan in doses of 2.5, 12.5 or 62.5 mg. per kg. (Table III). Pooled samples of serum from 3 rats were obtained at specific intervals between 2 hours and 10 days following zymosan administration at each level of injection.

TABLE III

Serum Properdin Levels of Rats Following Intravenous Administration of Zymosan (Time-dose Relationship)

Zymosan mg. per kg.	Serum properdin levels (units per ml.) at specified bleeding times following zymosan administration*					
	Hours		Days			
	2	24	3	5	8	10
2.5	12	30	40	30		30
12.5	6	18	20	35	40	30
62.5	6	6		25	30	40

* Individual serums from 3 untreated control rats contained 12 to 25 units of properdin per ml.

Following 2.5 mg., a fall from 25 units to 12 units occurred in 2 hours. At 24 hours, the serum properdin level rose to 30 units, at which level it was maintained for 10 days. After 12.5 or 62.5 mg. of zymosan, there was a fall in the serum properdin level to 6 units per ml. in 2 hours. At 24 hours after the injection of 12.5 mg. of zymosan, the serum levels rose to 18 units per ml., while following 62.5 mg., the serum level had remained at 6 units. Between 3 and 10 days after the injection of both 12.5 and 62.5 mg. of zymosan, the serum properdin levels rose to levels of 25 to 40 units per ml.

Successive doses of 8 mg. of zymosan per rat, given intraperitoneally 48 hours apart to 175 gm. rats, produced ascites after the fourth administration. The ascitic fluid was viscid and pale yellow. One week following the fourth and last injection, the ascites had disappeared spontaneously. Gross inspection of the peritoneal surfaces revealed prominent nodularity of the serosal surfaces of the intestine and parietal wall. Histologically, these nodules showed reticuloendothelial hyperplasia of lymph nodes and lymphoid aggregates. PAS stains of these nodules for mucopolysaccharide were negative. Rats kept for 30 days following the last introduction of zymosan were apparently healthy and grew normally without recurrence of ascites.

Serum properdin levels were determined on alternate days, between

the days of administration of 8 mg. of zymosan per rat. There was a fall in serum properdin level to 25 per cent of the control value 24 hours after the initial injection. Subsequent titers determined on serum collected between the second, third and fourth injections of zymosan were within the range of the untreated controls and apparently unaffected by the zymosan treatment.

Effect of Administration of Zymosan on Resistance of Mice to Klebsiella pneumoniae Infection. As has been shown, an appropriate single dose of zymosan, administered parenterally in mice, elevates serum properdin titers for periods up to 10 days. In published[2] and unpublished experiments,[13] it has been demonstrated that mice receiving zymosan intraperitoneally or intravenously are protected during the following interval of increased serum properdin level against infection with multiple lethal doses of *K. pneumoniae*.[2]

Histologic examinations (to be reported later) of mouse tissues after the intravenous administration of zymosan and after serum properdin levels had returned to normal, revealed foci of intracellular PAS positive particles suggestive of retained zymosan. The possible protective activity of the retained zymosan was studied at various intervals between 1 and 12 weeks following a single intravenous injection (Table IV). Groups of mice received single doses of 0.1 mg.

TABLE IV

Effect of Zymosan Administered Intravenously at Varying Times Prior to Intraperitoneal Inoculation with Klebsiella pneumoniae

Zymosan mg.	Interval: Zymosan to challenge (weeks)	Survival (8 days after challenge) 0.5 ml. injected intraperitoneally	
		75 organisms	15 organisms
Control		4/15*	8/15
0.1 (25 mg. per kg.)	12	9/14	14/14
	8	4/9	8/8
	4	5/9	6/8
1.5 (125 mg. per kg.)	4	5/6	6/6
	1	13/13	12/12

* Data presented as survivors divided by total mice inoculated (S/T).

or 1.5 mg. of zymosan by the intravenous route. Additional groups of mice from the same shipment were held as controls. One, 4, 8 and 12 weeks later, all mice were inoculated intraperitoneally with a small number (15 or 75) of virulent *K. pneumoniae* organisms.

Control mice inoculated with 15 or 75 *K. pneumoniae* organisms had survival rates of 8 in 15, and 4 in 15 respectively. Of the mice infected

with 15 organisms at 4, 8 or 12 weeks following preparation with 0.1 mg. of zymosan there were survival rates of 14 in 14, 8 in 8, and 6 in 8 respectively. Of the mice prepared with 1.5 mg. of zymosan 1 or 4 weeks before infection, the survival rates were 12 in 12, and 6 in 6, against a challenge of 15 organisms. Against a challenge of 75 organisms, mice prepared with 0.1 mg. of zymosan 4, 8 or 12 weeks before infection, yielded 9 survivors in 14, 4 in 9, and 5 in 9. Bacterial challenge with 75 organisms in mice given 1.5 mg. of zymosan 1 or 4 weeks previously yielded 13 survivors in 13, and 5 in 6.

Serum Properdin Levels of CF-1 Female Mice and Their Augmentation by the Intravenous Administration of Properdin. Serum properdin levels of normal CF-1 mice vary from 8 to 12 units per ml. of serum. Augmentation of these levels was studied following the parenteral introduction of heterlogous purified properdin in untreated CF-1 mice.

Fifty units of purified human properdin were injected intravenously into each of a group of mice weighing 20 gm. (Table V). Two, 24, 48, and 96 hours later, the pooled serum of 5 mice was assayed for properdin. A second group of mice received 20 units of properdin each and was also bled at the above intervals. Five untreated mice were bled for control serum properdin levels.

TABLE V

Properdin Levels of CF-1 Mice Following Intravenous Administration of Human Properdin

Properdin administered (units)	Time following administration (hours)	Properdin (units per ml. serum)
0 (control	0 (control)	12
50	2	48
	24	18
	48	12
	96	12
20	2	24
	24	18
	48	12
	96	12

At 2 hours following injection, a serum properdin level of 48 units was found in mice which had received 50 units of properdin, and a level of 24 units in mice which had received 20 units of properdin. The level in the control animals was 12 units per ml. At 24 hours, serum properdin levels were 18 units and 12 units respectively. At 48 and 96 hours the levels of both groups of mice were 12 units, or the level present before the administration of properdin.

The Protective Effect of Parenteral Administration of Purified Properdin Against K. pneumoniae Infection in CF-1 Mice. Inoculation of mice with large numbers of *E. coli* organisms results in a marked fall in serum properdin levels and death of the mice (Table I). Conversely, the introduction of zymosan results in the elevation of serum properdin levels of mice and subsequent protection against lethal infection by *K. pneumoniae* organisms. Both of these observations sug-

gest a causal relationship between serum properdin levels and resistance to infection. To test this possibility directly, properdin (human) was injected into mice infected with *K. pneumoniae.*

Female mice received 60 units of purified human properdin intravenously either before or after inoculation with multiple LD_{50} doses of a 5-hour *K. pneumoniae* culture. One group of mice received the properdin 4 hours before inoculation, another group ¼ hour before inoculation, and the third group 2 hours after inoculation. Dilutions of a 5-hour culture of *K. pneumoniae* were introduced intraperitoneally into the properdin-treated and into a separate group of normal, untreated mice. The bacterial inoculations were carried out in sequential order corresponding to the order used in properdin administrations, to maintain exact time relationships between the introduction of properdin and the bacterial infection.

Control mice inoculated with a culture dilution containing 10 organisms yielded one survivor among the 16 mice infected.

TABLE VI

Protective Effect of Parenteral Administration of Human Properdin Against Klebsiella pneumoniae Infection in Mice

Properdin administration time (60 units) intravenously	Survivors (7 days after infection) Dose (organisms injected intraperitoneally)			
	30*	95	135	230
4 hours before infection	16/16†	9/16	8/16	7/15
¼ hour before infection	10/16	8/16	12/16	4/16
2 hours after infection	13/16	7/16	9/12	2/12

Infective Dose (organisms)	M.S.T.‡ (hours)
10	60
30	48
90	48
135	36
230	24

* LD_{50} of *K. pneumoniae* culture was less than 10 organisms in control mice.

† All data presented as survivors divided by total mice infected (S/T).

‡ Median survival time (M.S.T.) of control mice infected intraperitoneally with *K. pneumoniae.*

Similar survival rates were obtained following inoculation with 30, 95, 135, and 230 organisms in other groups of control mice. Thus, 10 organisms represented more than one LD_{50}. The properdin-treated mice receiving higher dosages of organisms, therefore, were challenged with at least 3 LD_{50} (30 organisms) and as much as 23 LD_{50} (230 organisms) (Table VI).

Mice prepared with 60 units of properdin intravenously 4 hours before infection with 30 organisms had 100 per cent (16 in 16) survivors. Infection with larger doses resulted in 7 survivors in 15 against

a challenge of 230 organisms. Properdin administered 15 minutes before infection resulted in 10 survivors in 16 with a 30 organism challenge; this fell to 4 in 16 with a challenge of 230 organisms. When properdin was administered 2 hours after infection, 13 in 16 mice survived the lowest challenge dose of 30 organisms as compared to 2 in 12 when inoculated with 230 organisms.

Discussion

It has been shown[1] that the serum properdin level in man as well as in many lower animals is relatively constant for each species. It varies between 1 to 2 units per ml. in the guinea pig and between 25 to 50 units per ml. in the rat. Since these levels are relatively constant and present normally, the demonstration of a special property of properdin in regulating host resistance, in part at least, required testing under special experimental conditions in which normal serum properdin levels were altered following either the administration of zymosan or purified properdin.

Polysaccharides isolated from yeast (zymosan), bacteria and certain mammalian tissues, have been shown to alter serum properdin levels[6,9] when introduced into animals parenterally. With the exception of bacterial lipopolysaccharides, these substances were well tolerated parenterally in a wide range of dosages. Landy[9] has shown that as little as 100 μg. of purified endotoxin (lipopolysaccharide) administered parenterally was lethal to mice. In contrast, detrimental effects were minimal to absent following relatively large doses of zymosan in normal mice or rats, although an initial depression of serum properdin level, followed by a secondary rise, was characteristically found. However, when large numbers of live *E. coli* were injected into normal mice, there was a marked, rapid, persistent fall in serum properdin levels, followed by death of the mice. The stroma of *E. coli* contains high molecular weight polysaccharides possessing properdin binding properties similar to zymosan. Inoculation with smaller numbers of live *E. coli* was not followed by death of the mice and resulted only in a transient fall and prompt return to normal of the serum properdin levels. Since Wardlaw[14] has shown that large numbers of gram-negative organisms are rapidly killed *in vitro* in the presence of the components of the properdin system, a similar reaction presumably could occur *in vivo* in mice following the introduction of live organisms. Killing and lysis of the live bacteria *in vivo* with the liberation of endotoxin, attendant tissue damage and release of active endogenous polysaccharide to contribute to further depression of serum properdin levels, could contribute to a fatal toxemia. The response of mice to the

introduction of smaller numbers of *E. coli*, resulting in a transient fall in serum properdin level, is comparable to the reaction to low dosages of zymosan; neither injection is accompanied by systemic effects.

These data suggest that maintenance or elevation of serum properdin levels constitute important factors in sustaining host resistance to infection. It is further suggested that polysaccharides, active in combining with properdin, constitute significant factors in the regulation of the serum properdin level. Thus, within 5 minutes following the intravenous administration of an appropriate amount of zymosan, the serum properdin level falls to a low level, and increased susceptibility to bacterial infection is manifest during this period.[7,8,11,13] The rapid fall in serum properdin level during this period probably reflects direct combining of properdin with the zymosan.

The elevations of serum properdin levels, sustained 48 to 96 hours after the injection of zymosan, were accompanied by increased resistance to infection.[2] Isliker[15] has also reported that following administration of zymosan to mice previously treated with radioactive carbon, the concentration of C^{14} rose sharply in the properdin fraction subsequently isolated from serum of these animals. No increase in C^{14} was found in the gamma or beta fractions[15] of the serums.

Mowry[16] in a study of the biologic activities of dextrans (low molecular weight polysaccharides), reported their retention in the reticuloendothelial system of rats for periods up to 6 months following parenteral administration. In the present studies, PAS positive particles were also identified in body tissues up to 3 months after a single injection of zymosan. Although serum properdin levels had returned to normal values, these mice showed substantial levels of resistance to challenges with multiple lethal doses of *K. pneumoniae* (Table IV). Similar results were obtained independently by Kiser,[17] using the same organism. Dubos[18] also observed increased survival in staphylococcus infections for periods up to 49 days, following the administration of pertussis vaccine and typhoid lipopolysaccharide.

Although these experimental observations demonstrate the development of nonspecific resistance to bacterial infection continuing for periods up to 3 months after a single injection of zymosan, the mechanism of this reaction is as yet poorly understood. The PAS positive masses observed within cells as long as 3 months after the injection of zymosan could represent potential sources of polysaccharide capable of elevating serum properdin levels rapidly upon their release. Thus, in another series of experiments, serum properdin titers were obtained 3 and 24 hours after total body irradiation with 600 r. in mice which had been prepared with zymosan 3 months earlier.[19] These mice

showed serum properdin levels of 12 units per ml. immediately before irradiation. Three hours after irradiation, the serum level was 18 units per ml. and after 24 hours, 24 units per ml. Serum properdin titers of mice not similarly prepared with zymosan exhibited a fall of up to 60 per cent in serum properdin level within 24 hours after irradiation. One interpretation of these observations might be that the injury from total body irradiation or infection may serve to release active stored polysaccharides of endogenous origin and thus elevate serum properdin in the same manner described in the present study.

Evidence that the level of circulating serum properdin bears a direct relationship to the level of host resistance to infection was shown by the protection of mice receiving parenteral administration of purified human properdin and challenged with *K. pneumoniae* organisms. The time-dose relationships of the infection to properdin administration did not markedly influence the outcome of the infection. Protection was obtained when properdin was injected either before or after inoculation. Protection was significantly reduced only when large numbers of organisms, representing more than 20 lethal doses, were introduced following the fixed dose of 60 units of properdin. Thus, the limits of protection obtained were relative to a fixed number of units of properdin, as compared to a rising challenge dose of highly virulent organisms. However, protection with properdin against multiple lethal doses of *K. pneumoniae* exceeds that generally obtained following the use of specific antibody. When sufficient amounts of both homologous and heterologous properdin are available, the effect of other dosage schedules can be investigated.

Summary

Intraperitoneal inoculation of mice with large numbers of live *E. coli* resulted in a marked fall in serum properdin level and death of the mice. The parenteral introduction of zymosan into mice and rats resulted in an initial fall followed by a sustained rise in serum properdin levels.

Increased host resistance to infection with *K. pneumoniae* followed elevation of serum properdin resulting from the parenteral administration of zymosan.

Increased nonspecific host resistance to infection with *K. pneumoniae* in mice persisted for periods up to 3 months after a single injection of zymosan, despite the return to normal of serum properdin levels.

Purified human properdin introduced intravenously in mice was rapidly destroyed in 2 to 24 hours.

Purified human properdin introduced intravenously into mice 4 hours before or 2 hours following intraperitoneal inoculation with *K. pneumoniae* resulted in markedly increased protection of the mice.

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ADDENDUM

Additional observations on the interrelationships of properdin to resistance to experimental infection in mice were made after submitting the foregoing manuscript for publication. Purified human properdin derived from serum by chemical methods not requiring the use of zymosan adsorption has been prepared.* Limited amounts of this material were available for use as a protective agent in mice inoculated with *K. pneumoniae* in a manner similar to that described in the foregoing report. A group of 64 mice was divided into 4 subgroups of 16 mice each, and the subgroups were challenged with 2, 5, 10, or 25 lethal doses of *K. pneumoniae*, 2 hours after the intravenous administration of 60 units of properdin. Seven days after infection, the survival rates of these mice were 9 in 16, 11 in 16, 10 in 16, and 12 in 16, respectively. Survivors of mice treated with zymosan-adsorbed properdin and saline controls were similar to those previously reported. These results indicate that properdin isolated chemically was as effective as zymosan properdin in protecting the mice from infection.

TABLE VII

Comparative Survival Rates Among Mice Treated with Properdin in Two Forms, and Control Mice, Inoculated with Klebsiella pneumoniae

Time	No. of lethal doses, K. pneumoniae	Active properdin (60 units)	Boiled properdin (1 unit or less)	Saline control
4 hours after properdin	2	14/16*	12/16	0/16
	5	13/16	13/16	2/16
	10	11/16	11/16	3/16
	25	14/16	14/16	1/16
2 hours before properdin	2	16/16	15/16	7/16
	5	13/16	13/16	4/16
	10	12/16	13/16	0/16
	25	6/16	2/13	0/16

* All data presented as survivors divided by total mice infected (S/T).

Purified human properdin prepared by the zymosan method was inactivated by heating at 100° C. for 30 minutes. *In vitro* assay following heating showed less than one unit per ml. of properdin activity as compared to an original titer of 60 units per ml. One-half ml. of heat-inactivated properdin was administered intravenously to mice either 4 hours before or 2 hours after intraperitoneal inoculation with

* Pennell, R. B.; Rothstein, F.; Surgenor, D. M.; Eyquem, A., and Todd, E. W. Isolation of properdin from human plasma. *Proc. Soc. Exper. Biol. & Med.*, 1957, 96, 273-277.

K. pneumoniae. Suitable saline control animals and active properdin controls (60 units) were inoculated simultaneously. Survivors of these treatments 7 days after infection are shown in Table VII.

Inspection of the data reveals that protection was afforded the infected mice by treatment with both heated and undenatured properdin solutions. These observations are presented as preliminary findings only. Further studies are under way for the purpose of explaining the relationships which may exist between the protective capacity of active and heat-treated properdin solutions.

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MYELOLIPOMA IN THE ADRENAL CORTEX

(MYELOADIPOSE STRUCTURES)*

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The terms heterotopic bone marrow and extramedullary hematopoiesis, often used synonymously, are associated in the physician's mind with anemia or other hematopoietic disorders. Circumscribed lesions of this nature, called myelolipomas, are seen on occasion in the adrenal cortex at necropsy. While authors of some papers dealing with these lesions record the presence of anemia, others note its absence, and even those reporting impressively large lesions pass in silence over the problem of pathogenesis. Therefore, a discussion of structures similar to bone marrow but not associated with a manifest disorder of the blood-forming systems seems indicated. A well known modern textbook of pathology says of the adrenal myelolipoma: "There may be no anemia and no other foci of extramedullary hemopoiesis,"[1] thus creating the impression that they are present in most cases.

Circumscribed bone marrow-like structures in the adrenal were first recognized by Gierke[2] in 1905, and were given the generally accepted name myelolipoma by Oberling[3] in 1929. Biressi[4] used the more comprehensive designation, myeloadipose structures. The statement that Arnold reported a myelolipoma in 1866 is based on an error in reference. The "adrenal lipomas" of Mattei (1883),[5] Brüchanow (1899)[6] and Marchetti (1904)[7] probably were myelolipomas. I have never seen a lipoma of the adrenal or read a convincing description of one.

MATERIAL

Fifty myelolipomas from the Armed Forces Institute of Pathology were studied and compared with an approximately equal number reported in the literature. In addition, the adrenal glands removed at 117 necropsies, which I performed at the Veterans Hospital, Topeka, Kansas, were studied especially for fat cells and so-called lymphocytes in the cortex. Added to these were the adrenal glands from 23 burn victims, those from 29 cases of sudden traumatic death and 29 cases of starvation, and finally more than 100 from domestic and captive wild animals.

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General Observations

Grossly, most myelolipomas resemble lipomas (Fig. 1), but when myeloid tissue is abundant, the cut surfaces may be gray or red. Hyperemia and hemorrhage can alter the appearance profoundly. Various queer shapes result from the location and expansion of the lipoma-like masses (Figs. 2, 15). The myelolipoma may arise in cortical tissue, irrespective of its location, and, since cortical tissue may be present between the layers of the capsule or outside the capsule in the periadrenal fat, myelolipomas may also arise there (Figs. 4, 5). One cannot determine definitely which of the three layers of the cortex is the site of origin, because sharply localized, sufficiently small lesions are rare, but the inner half of the *zona fasciculata* seems to be the principal site of myeloadipose changes. An extracapsular myelolipoma need not have originated in an outer layer or a central one in an inner, because the three layers can be inverted in the central cortex as well as in portions of cortex which extend beyond the capsule. When a large fatty nodule reaches the capsule, leaving little or no remnant of cortex beneath it, its appearance may be puzzling, especially when periadrenal fat is abundant (see legend to Fig. 12). A myelolipoma situated between the layers of the capsule or in the periadrenal fat tissue may have arisen in any of the three zones, since cortical tissue which extends into the periadrenal fat may lose its regular layering.[8] Indeed, pigmented cells of the *zona reticularis* may be found in the outer portions of extracapsular cortical tissue.

The lesions exhibit all gradations from almost entirely adipose to almost entirely myeloid tissue (Figs. 3, 7, 8, 13, 15). The ratio of fat tissue to myeloid tissue is difficult to judge when, as I have seen in two cases, the myelolipoma is formed in cortical tissue which has grown through the capsule into the periadrenal fat (Fig. 5). It may be impossible to decide which is original periadrenal fat and which the fat tissue that developed in the extracapsular cortex as part of the myelolipoma. Small and medium sized nodules may be almost entirely fatty or entirely myeloid. The cellular marrow often is peripherally located, while the fat is central; the reverse may also occur (Figs. 8, 15).

Connective tissue plays a minor role, and no continuous fibrous layer separates the myelolipoma from the surrounding cortex; thus, it does not have a true capsule. Compression and condensation of surrounding tissue may result in pseudocapsule formation around a nodule, or unequal growth may produce apparent septation within it (Fig. 15).

The gross appearance of a circumscribed myelolipoma often does not differ from that of a lipoma, and the diagnosis "lipoma of adrenal cortex" appears in necropsy protocols and in the literature.[5-7] But large lipomas are lobated, whereas large myelolipomas are not.

In the absence of a capsule, there is no sharp dividing line between the cells of the cortex and those of the myelolipoma. The contour of the lesion is wavy (Figs. 7, 8, 13) or irregular, with fat cells extending into the surrounding tissue, and portions of cortex protruding into the myelolipoma (Figs. 7, 13). Bits of well preserved cortical tissue occasionally are surrounded on all sides by the bone marrow-like tissue (Figs. 21, 22). The surrounding cortical cells may be either unaltered or compressed, without relation to the size or cellular composition of the myelolipoma. Perhaps it does not expand steadily, and the presence or absence of compression depends upon the phase of growth at the time of death.

Some myelolipomas, notably those containing much myeloid tissue, approach a spherical shape, are sharply outlined, and compress the surrounding tissue, obviously as the result of concentric expansion. Less regular shapes and irregular outlines probably indicate pluricentric origin and growth by apposition. Evidence of both mechanisms may occasionally be seen in the same specimen. Pressure alone cannot explain the thinning of the cortex. Narrow strips of fat tissue stemming from a myelolipoma may even extend to the capsule through a thick layer of otherwise intact cortex.

Forty-three specimens could be measured. None were spectacularly large; the largest measured 4 cm.; 5 were between 2 and 4 cm.; 18 between 1 and 2 cm.; 11 between 0.5 and 1.0 cm. The remaining 9 were slightly less than 0.5 cm. in diameter. Myelolipomas may grow very large. The bilateral adrenal masses Holliday[9] observed measured 6 by 5.5 by 4.5, and 4.5 by 4.5 by 2.5 cm., respectively. The greatest diameter in the case reported by Richardson[10] and in that of de Navasquez,[11] was 8 cm. In van Dam's[12] case the myelolipoma was twice the diameter of the normal kidney. Even these unusually large tumors did not give rise to symptoms. The two almost entirely cellular myelolipomas in the material described here were of medium size. Large ones as well as small ones were wholly or almost entirely fatty.

Some reports have stated that no medulla could be seen in the sections. In one case (Barten,[13] case 2), the whole adrenal was examined in step series, but no medulla was found. Only a small area of medulla was identified in one of my cases, although more than 20 blocks were cut.

Microscopic Observations

The myelolipoma consists of fat cells and of cells that are identical with or similar to bone marrow cells, and lymphocyte-like elements, but the variations in numbers, arrangement, and ratio of the components may make it difficult to decide whether or not the diagnosis should be myelolipoma. Numerous minute foci may represent diffuse myelolipomatosis; on the other hand, a group of fat cells with a dozen "lymphocytes" and a few cells resembling myelocytes should not be called myelolipoma. A regularly convex contour and compression of the surrounding tissue may serve as distinguishing criteria, and generally the diameter of such a lesion will be 4 mm. or more. In a group of such borderline cases, the frequency of irregular adenoma-like cortical nodules was impressive. The most diffuse myeloadipose infiltration was found in a patient with profound endocrine disturbance (diabetes, cataracts, hypotension, and a thecoma of the ovary, AFIP Acc. 719645). The lipid content of the surrounding cortical cells does not differ from that of the cortex in general.

The cells in the myelolipomatous area, other than the hemic and the well defined fat cells, are of bewildering variety, and this, together with the similarity between fat cells and lipid-filled cortical cells, has led to conflicting interpretations. Some areas (Figs. 16, 20) are occupied mainly by elements that approximate the fat cells in size but have a fairly compact cytoplasm. Bands, networks and islands are formed by similar cells of varying sizes and densities, with one thing in common: their nuclei are irregularly round and are not pushed to the periphery as are those of fat cells (Fig. 16). The gussets between some of the fat cells contain single large cells, each with a bizarre nucleus (Fig. 19). In addition, there are cells with obvious degenerative changes, vacuolar and otherwise (Figs. 16, 20), too varied for detailed description or illustration. All of these represent altered adrenal cortical cells.

The vascularization of the myelolipoma is difficult to evaluate, because collapsed capillaries may be unrecognizable. The accumulation of fat in the reticulum may narrow the capillaries by compression or stretching. The vascular channels seem to be less numerous than in the neighboring adrenal cortex, an observation already made by Gierke.[2]

A search for the sources of the bone narrow-like tissue in myelolipoma must take into account its two components, fat cells and blood-forming elements.

The Fat Cells

Fat cells, singly or in groups, often occur in the human adrenal cortex but have received little attention, perhaps because they are not known to be related to disease. Gierke,[2] in describing them, expressed surprise that they had not been mentioned before. Paunz,[14] who studied the adrenal glands in 500 necropsies, observed them "repeatedly." Gossmann[15] noted fat cells, varying from single cells to 15 or 30 in one section, in 21 of 150 glands; they were absent in children, rare in persons less than 40 years of age, but frequent in those more than 70. This increased incidence with advancing age was confirmed by Stieve[16] in a detailed study of 511 adrenal glands from people who had died by violence and in whom there was no evidence of disease at necropsy. He noted that fat cells appeared when the gonads became atrophic under conditions of prison life. In young women who had had amenorrhea for a year or longer, fat cells were fewer than in women more than 35 years of age. These observations were corroborated in the material examined in this study. Furthermore, no fat cells were found in single sections of adrenal from 29 cases of sudden traumatic death of young men.

Little, if anything, seems to be known about the occurrence of fat cells in the adrenal cortex of animals. I found none in one or more sections from several mammalian species (10 chimpanzees, 7 macacus monkeys, 46 cows, 14 sheep and 34 cats). They were present, however, in the adrenals of 2 of 27 dogs and 1 of 2 opossums, and singly or in small groups in 6 of 11 rabbit adrenals studied in series.

The origin of the fat cells in the adrenal cortex is a much debated topic. Single fat cells in a lipid-rich cortex are not conspicuous because they resemble the cortical cells, and some authors have tacitly or expressly assumed that they were transformed cortical cells.[16-20] This is in contradiction to the prevailing idea that the fat cell is a modified reticulum cell[21,22] or, perhaps, a modified fibroblast.[23] The transformation into fat cells of highly differentiated adrenal cortical cells seems as improbable as would such a metamorphosis of parenchymal cells in a severely fatty liver. The cortical cells between the fat cells of the myelolipoma, or between grouped fat cells in the cortex, disintegrate and disappear in the same way as do the epithelial elements in adiposity of the pancreas or parathyroid gland. While lipid-rich adrenal cortical cells may mimic fat cells, the true fat cells can be recognized by a detail of their nucleus, namely, the *Lochkern*. This descriptive

term, coined by P. G. Unna[24] in 1895, means a nucleus with a hole. In most fully developed fat cells, the nucleus is indented by a small lipid droplet, which is continuous with the vacuole that fills the remainder of the cell. Continuity with the larger vacuole is not readily seen in sections; hence, the misleading name. As noted by Omelskyj,[25] the indented nucleus occurs in fat cells in myelolipoma and in the adrenal cortex. It does not appear in lipid-filled cortical cells. In good paraffin sections, the punched-out defect in the nucleus was found almost as easily in the fat cells of myelolipoma as in those of the periadrenal fat, and it was only a matter of prolonging the search to find it in other fat cells scattered through the cortex. The presence of these characteristic nuclei proves that the fat cells of myelolipoma are true fat cells and not transformed cortical elements. Further proof lies in the condition of the cortical cells surrounding the myelolipoma. These often contain little lipid, which would not be the case if the myelolipoma had arisen by fatty transformation from them.

The resulting histologic pattern of the cortex is comparable to that of the adipose parathyroid gland or pancreas, but the presence of myeloid cells or "lymphocytes" in the myelolipoma indicates a biologic difference. Even severe adiposity of parenchymal organs does not lead to the formation of circumscribed lipoma-like structures comparable to the myelolipoma.

The close relationship between fat tissue and hematopoietic tissue has its basis in their common origin from reticulum. The simultaneous or successive appearance of fat and hemic cells would be inexplicable if the fat cells were derived from the cortical cells and not from the reticulum.

The So-called "Lymphocytes"

In searching the adrenal cortex for a source of the blood-forming elements, one automatically considers the cells often present in abundance, for which, unfortunately, no fitting name exists. They have been called lymphocytes,[26] round cells,[14,27] small mononuclear cells, polyblasts, and *cosidette cellule rotonde*.[4] For the sake of convenience, I shall call them "lymphocytes" (which many of them are not). The interpretation and designation of these cells in the literature of 7 decades reflect the changing opinions about origins and relationships of circulating and fixed cells. The paucity of illustrations may indicate uncertainties of interpretation. The idea that these cells stem from the reticuloendothelial cells will hardly be disputed today. They were mentioned as early as 1887,[27] and have been investigated much more than the fat cells, perhaps because they appear alien to the adrenal cortex and supposedly are related to infection. All authors agree that

they are frequent in the adrenals of adults; the incidences given vary from 17 to 100 per cent. Some authors consider their presence in the adrenal normal. E. Thomas[28] found them in 38 of 40 cases, in each of which he examined 50 sections from 6 or 8 blocks. In the 2 cases in which these cells were absent, death was caused by execution in one, and septic tracheitis, probably a disease of short duration, in the other. Stieve[16] found them only in persons more than 50 years of age, except for a few women with amenorrhea between 35 and 50. He found them regularly in old people. I found the "lymphocytes" at necropsy in 77 per cent of 117 adult males at the Veterans Hospital in Topeka. In these, serial sections were prepared from some blocks and single sections from others. They were more frequent in older patients (34 of 41 more than 60 years of age; 9 of 10 more than 75). The number and size of the foci bore no relation to the weight of the adrenals or to the necropsy findings. It may be anticipated here that the incidence in relation to age was the same in the cases of myelolipoma, and as far as one can judge from the literature, this has been the experience of others also. On the other hand, among the 29 cases of sudden traumatic death in which fat cells were not seen in the adrenals, neither were "lymphocytes."

The erroneous belief that these cellular foci were related in some way to infection persisted until recently, in spite of Landau's[29] correct observation in 1915 that they were not associated with infection. The older literature often mentions "heaps of lymphocytes"[30] and their possible relationship to the disease that caused death, but these assumptions do not appear well founded today. The cellular foci were not more frequent in the old necropsy material with its many infectious and septic cases than in that of the last two decades in which chronic noninfectious diseases have been prevalent. Only in cases of sudden death without disease are they absent or rare. This indicates that the cause is not infection but disease in general. One may assume, then, that common disturbances in the organism cause fat cells and "lymphocytes" to appear in the adrenal cortex, while some unknown, uncommon ones result in the formation of myelolipoma.

Information about the occurrence of "lymphocytes" in animal adrenals is contradictory, possibly because of the ill-defined nature of these cells. In the present investigation a few were found in a series of sections of rabbit adrenals and in single sections from cattle and dogs; none were observed in 24 rat adrenals.

Single fat cells or small clusters of them may appear in cortical tissue which is normal except for slight compression of adjacent cells (Figs. 6, 9). Even an isolated fat cell may be accompanied by

"lymphocytes" (Fig. 11), and larger and more compact groups of fat cells often contain considerable accumulations of these elements (Fig. 10). At medium magnification, collections of this nature, with both densely cellular and fatty portions, appear as myelolipomas (Fig. 8), but if the so-called lymphocytes were really lymphocytes, this resemblance would vanish under higher magnifications. However, as the indecisive nomenclature suggests, the pattern of these cells is varied. Ordinary "cellular foci" in adrenals at necropsy often contain cells with abundant cytoplasm and chromatin structure resembling that of myeloid elements (Figs. 14, 17, 23). These cells may show cytoplasmic granulations when stained with Giemsa stain. Some cells with very compact nuclei probably represent erythroblasts, and origin from sinusoidal lining cells is suggested by dark-staining, swollen endothelial nuclei (Fig. 14). The conviction that such myeloid elements originate *in situ* becomes firmer from careful study of microscopic sections than from viewing a limited number of illustrations.

Incidence

The myelolipoma is not such a rarity that every single case will find its way into the literature. Thus, its incidence cannot be truly gauged from reports which are available. McDonnell,[21] who reported 4 cases, gives an incidence of 0.2 per cent for a series of 2,000 necropsies, which probably included children and infants. Mattei's[5] 5 "adrenal lipomas" occurred in 1,951 necropsies. Biressi[4] in 1954 listed 54 cases, some of which, in my opinion, were not myelolipomas but represented bone marrow formation within ectopic bone.[22-36] Bone marrow within bone does not belong in the category of myelolipoma. Ossification occurs in the adrenal gland, notably after hemorrhages and after necrotizing and chronic inflammatory processes. The fact that such lesions have been called myelolipomas[24,37] creates confusion. Generally it is easy to distinguish between bone that contains marrow and a mass of myeloid tissue that has formed a few spicules of bone. The two reported myelolipomas in infants[25] represented bone marrow within bone. It is important to remove these two cases from the category under discussion because, so far, true myelolipoma has not been observed before puberty. The case of Vera Hirschfeld, which appears in the lists of Biressi[4] and others, cannot be verified (Hirschfeld's paper does not mention it[38]).

The youngest patient with myelolipoma in the AFIP series was 17 years old, the oldest 93. Twenty-seven of the 50 were in the fifth and sixth decades; 33 were between the ages of 46 and 65; 39 between the ages of 36 and 65. When these figures are compared with the death

rates for different age groups of adult males (U. S. census for the year 1950), the occurrence of myelolipoma appears as 1 in 7,600 for the age group from 36 to 65, with practically no difference between these three decades, while the frequency for all other decades is 1 in 41,000. There is no way of determining how long a myelolipoma found at necropsy has taken to develop.

There was no correlation between the myelolipoma and disturbances of the hematopoietic apparatus, despite the resemblance to bone marrow. The 50 cases from the AFIP included only one instance of leukemia and one of doubtful hyperplasia of bone marrow. The relative frequency of the diseases accompanying myelolipoma was not unusual. Nine cases of cirrhosis of the liver are perhaps more than should be expected, but I have observed a similar preponderance of cirrhosis in obviously unrelated lesions such as focal arteritis and pituitary necrosis.

There was no indication of a relationship to hypertension.

Endocrine Correlations

Twenty-eight of the 50 patients studied were obese, 11 of them severely so. The frequency of obesity appears even more striking when one considers the 22 cases in which the diameter of the myelolipomas was 1 cm. or more. Of these 22 patients, 15 were obese, 7 severely so. This predominance of obese patients might lead one to suspect an alteration of fat metabolism.

Significant endocrine disturbances were present in 9 of the 100 cases. Auvray[39] found large bilateral myelolipomas in a 72-year-old pseudohermaphrodite who had small internal sex organs, no vagina or vulva and a large, partly perforated clitoris. A schizophrenic female intersex, 58 years of age, also had large bilateral myelolipomas.[40] Bilateral myelolipomas (size not given) were found in a 49-year-old man with severe pluriglandular disease.[41] Four weeks after removal of a "fist-sized" adrenal adenoma, a young woman who had become virilized was normal again and menstruated.[42] This adenoma, which is not described in detail, contained much bone marrow. Sternberg,[43] without giving details, mentioned a myelolipoma in a contracted adrenal, and Paul[26] found a myelolipoma the size of a pigeon's egg in a woman who for one year had shown the classical symptoms of Addison's disease. In two instances,[44,45] myelolipomas were associated with extreme obesity, splanchnomegaly without acromegaly, and sudden death. The "adenoangiolipoma" which Letulle[46] observed in a hermaphrodite obviously must have been, at least in part, myelolipomatous. I would not draw the conclusion from these cases that myelolipoma

exerts an endocrine influence, but I believe that the adrenal cortex, in the presence of severe endocrine disturbance, is more likely to react with the formation of myelolipoma. The possibility of participation in a clinical syndrome is shown, however, by the cases of Schmidt[44] and of Plaut[45] cited above. A diffuse infiltration of the adrenal cortex by fat cells and myeloid elements may fall into a similar category (male, 39 years, severe Cushing's syndrome, AFIP Acc. 727046). It should be noted also that 3 of the 8 patients with bilateral myelolipomas had severe endocrine disorders: one intersex,[40] one pseudohermaphrodite,[39] one man with pluriglandular insufficiency.[41]

Pathogenesis

The life history of the myelolipoma is unknown. It has not been found before puberty, but appears with increasing frequency in older age groups. It is not known in what phase it is more fatty or more myeloid. Oberling[8] thought that the myelolipomas which were predominantly fatty represented the older lesions, but the study of many cases has convinced me that the fat cells may just as well precede the hematopoietic elements.

While attempting to understand the presence of myeloid cells in the adrenal cortex, one wonders whether such cells are found there in the wake of definite diseases or injuries. The only instance encountered is death after burning. Delarue and Monsaingeon[18] have found *"des îlots myeloides indiscutables"* in 3 of 8 burn victims; these 3 had died between 3 weeks and 3 months after the accident. The myeloid cells, in places, were close to fat cells, and a fine reticulum "completed the analogy with bone marrow tissue." The authors did not succeed in reproducing this adrenal lesion in the rat or the rabbit.

The adrenal glands from 22 of the 23 nonseptic burn cases were not unusual; the fact that necrosis and thrombosis were seen in one is not astonishing.[47] The adrenal of one case, however, was remarkable. The patient, a 20-year-old pregnant woman, died 8 days after a gas explosion which had burned 80 per cent of her body surface. The leukocyte count on admission was 10,150, but on the sixth hospital day it had fallen to 2,400. On the following day it rose again to 11,150. The bone marrow at necropsy showed slight hyperplasia and maturation arrest, consistent with early agranulocytosis. Myeloid reaction was slight in the liver and spleen but severe in the adrenal cortex. This may have been caused by the same unknown factors that were operative in the 3 burn cases cited above.[18] Gormsen[48] observed a similar distribution of hematopoiesis in a woman who had died of anemia from metrorrhagia.

Formation of fat tissue and hematopoiesis may occur simultaneously, not only in the embryo and in bone marrow, but also in organs of the adult under conditions other than anemia or infection.[49-53] The one well established point in the etiology of the myelolipoma is that its precursors, the cortical fat cells and "lymphocytes," are absent in persons killed while in good health. No significant correlation with a single disease exists. The fact that in 3 instances the myelolipoma contained foreign bodies suggests the possibility of local trigger mechanisms.[54] Injection of fat tissue juice into the adrenal cortex of rabbits, and implantation of a piece of omental fat, with its blood supply preserved, into the adrenal of a dog did not result in formation of fat cells or hemic cells.*

The question of how far the functional activity of the adrenal cortex can influence the formation of myelolipoma can hardly be answered by the study of routine sections. Cortical nodules seldom accompanied a circumscribed myelolipoma; they were more often found in conjunction with diffuse myeloadipose or myeloid infiltration. In animals, myeloid cells have occasionally been limited to cortical adenomas. In man, in the absence of myelolipoma, only a few myeloid cells appear in the adrenal cortex; one must hunt for these among the "lymphocytes."

In the adrenal cortex of man and probably of the rabbit, fat cells predominate over myeloid cells, while the reverse obtains in the dog and in cattle. Such observations reflect the labile balance between fat tissue and hematopoietic tissues.

Related Extra-adrenal Structures in Man

Finally, the question arises as to how far the adrenal myelolipoma is related to similar tumor-like bone marrow structures occasionally found in retroperitoneal or retropleural tissues.[55-66] They have not been seen in the places in which ectopic nodules of adrenal cortex are frequent, namely the broad ligament and the spermatic cord. Assuming that some general condition was responsible for the formation of the ectopic structures resembling bone marrow, one would expect such lesions to accompany myelolipoma, but in only 1 out of 14 cases of this nature is an adrenal myelolipoma mentioned, and that a small one.[61] One of these case reports includes a statement that the adrenal gland was normal; the others do not mention it.

In 4 of 12 cases,[55-66] the hematopoietic system was normal and there was no anemia; in 3 there is no statement about anemia; in 2 there was carcinoma with anemia; in 2, pernicious anemia; and in 1, partial

* Experiments carried out with R. M. McCully.

osteosclerosis (but no mention of blood findings). These bone marrow-like structures thus are more often associated with anemia than is the case with myelolipoma, and this indicates that some of the conditions which lead to adrenal myelolipoma must be looked for in the adrenal cortex itself. The sex distribution is significantly different from that of myelolipoma, since 13 of the 14 patients were female.

Tentative Classification of Myeloadipose Structures

Three entities should be kept separate: the myelolipoma, extramedullary hematopoiesis, true bone marrow.

The myelolipoma consists of myeloid cells and fat cells and contains reticulum only in the fatty portions (Figs. 21, 22). The absence of reticulum and sinusoids stamps the myelolipoma as something different from bone marrow, a fact not to be overlooked in spite of the apparent similarity. Since the myeloid cells are not situated in reticular sinusoidal structures, it is hard to see how blood cells formed in such an area can enter the general circulation. Certainly there can be no controlled releasing and holding mechanisms as in normal bone marrow.

Extramedullary hematopoiesis consists of myeloid cells without fat cells. As far as I can judge, no reticulum is formed in such areas, and, in large foci at least, pre-existing reticulum may be destroyed. The simple fact that in extramedullary hematopoiesis bone marrow is not formed[48] should always be kept in mind.

True bone marrow occurs in the adrenal gland when calcification has led to bone formation. Its reticulum is like that of normal bone marrow.

Conclusions and Summary

Myelolipoma of the adrenal cortex, although recognized for half a century, remains unexplained. It has been found only in man. It consists of adult white fat tissue and varying numbers of hemic cells. Some of the latter are myeloid elements, others resemble lymphocytes. The lesion occurs most frequently in middle life, has no sex predilection, and has not been described before puberty. It bears no relation to anemia or other disturbances of the hematopoietic system and has nothing to do with compensatory extramedullary hematopoiesis. It has no relation to any single disease, and no single endocrine influence is known. The fact that a number of patients, notably with large and bilateral myelolipomas, had severe endocrine disturbances suggests that the abnormal adrenal cortex may be more prone to form myeloadipose structures.

The simultaneous or alternating formation of fat cells and hematopoietic cells from the reticulum of the adrenal cortex might be analogous

to processes which occur during embryonic development, but the myeloadipose structures do not stem from embryonic rests. In the myeloid portions of the myelolipoma the reticulum is destroyed. Thus, it cannot be said that the myelolipoma is bone marrow; it merely resembles it.

The myelolipoma represents a local intensification of a more diffuse process which is present at necropsy in the adrenals of a high percentage of adults, namely, diffuse or focal infiltration by fat cells and "lymphocytes." These, however, are absent or very sparse in the adrenals of people who have been in good health before death, and thus represent a reaction to disease in general, without relation to a single disorder or group of disorders. The assumption that they are caused by infection is disproved by statistical studies which show that the lesions are no less frequent in the necropsies of today than they were decades ago when infectious diseases were much more prevalent. The absence of fat cells and hemic cells in the adrenals of healthy people and their almost equal frequency in a wide variety of diseases point to general causative factors. There must be a link between disease in general and the tendency of the reticulum of the adrenal cortex to form fat and hemic cells.

The adrenal cortical cells in the area of the myelolipoma disintegrate and disappear in the same way as do epithelial cells in adiposity of other parenchymal organs. Cortical cells are not transformed into true fat cells. The true fat cells in the adrenal cortex, as elsewhere, can be recognized by the characteristic indented nucleus, the *Lochkern*.

Myelolipoma in animals has not been recorded. That the myeloid cells in the adrenal cortex of animals represent a related phenomenon is a matter of speculation. Masses similar to bone marrow, found in retroperitoneal or retropleural regions, differ from myelolipomas by their association with blood disorders, especially anemia, and by their apparent limitation to the female.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

All illustrations are from men (or animals) without known hematologic disease or important infection. Illustrations were made from sections stained with hematoxylin and eosin unless stated otherwise.

FIG. 1. AFIP Acc. #633142, Neg. 55-8768. Spherical myelolipoma, 1 cm. in diameter. The myelolipoma has the gross appearance of lipoma unless myeloid tissue is abundant or there is hemorrhage. Gross specimen. × 2.

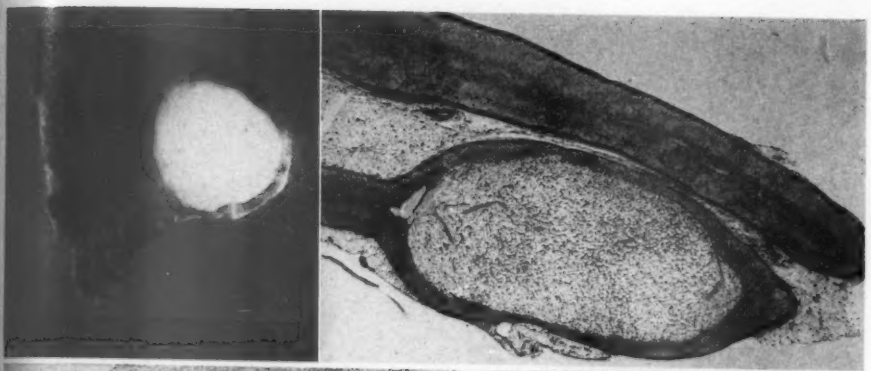
FIG. 2. AFIP Acc. #629321, Neg. 56-8712. A myelolipoma which obviously has expanded evenly in all directions. × 4½.

FIG. 3. AFIP Acc. #196568, Neg. 55-16455. A 7 mm. nodule consisting almost entirely of cellular marrow. The surrounding cortical tissue appears considerably stretched. × 10.

FIG. 4. AFIP Acc. #721469, Neg. 56-9457. A mostly myeloid myelolipoma situated between the layers of the capsule. × 9½.

FIG. 5. AFIP Acc. #323582, Neg. 55-12865. Cortical tissue protrudes deeply into the periadrenal fat tissue, which contains two round, partly myeloid, partly fatty, areas of myelolipoma and a less distinct, diffuse cellular infiltration. There is no myelolipoma within the normal boundaries of the adrenal cortex. × 10.

FIG. 6. Four partly confluent fat cells in adrenal of an adult female rabbit. They were the only ones in a large area of cortex. × 185.



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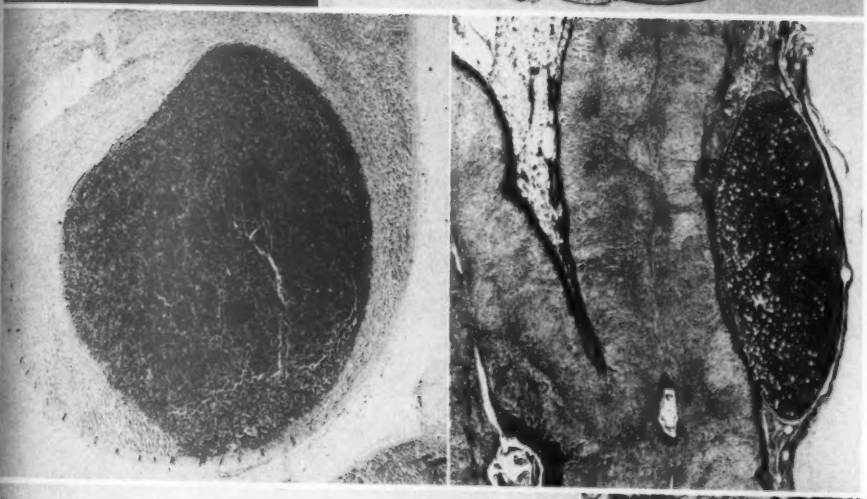

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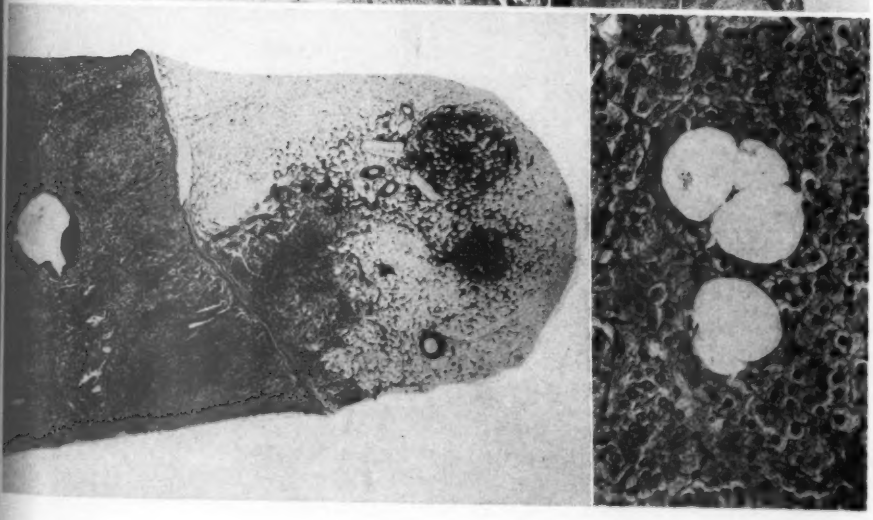

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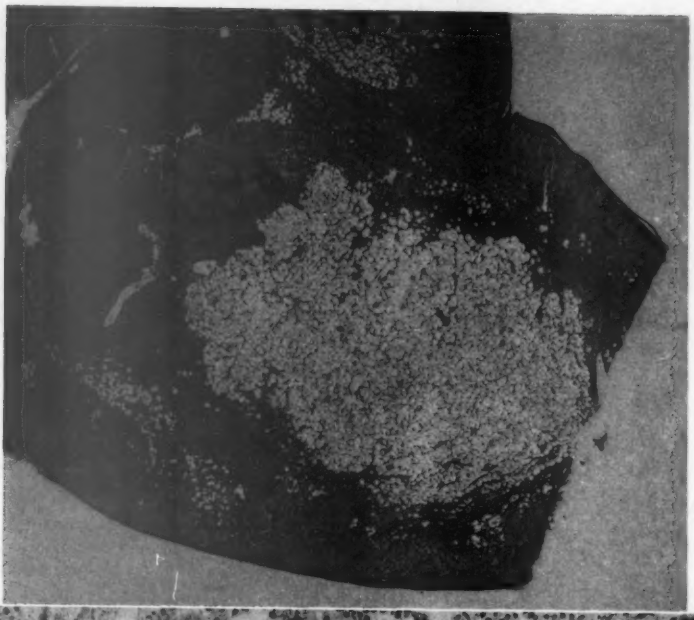
FIG. 7. AFIP Acc. #724046, Neg. 56-22521. A myelolipoma-like area and also diffuse infiltration by fat cells. Other cellular elements cannot be recognized at this magnification. × 9.

FIG. 8. AFIP Acc. #665671, Neg. 5156-2461. A fatty and cellular area resembling myelolipoma. × 90.



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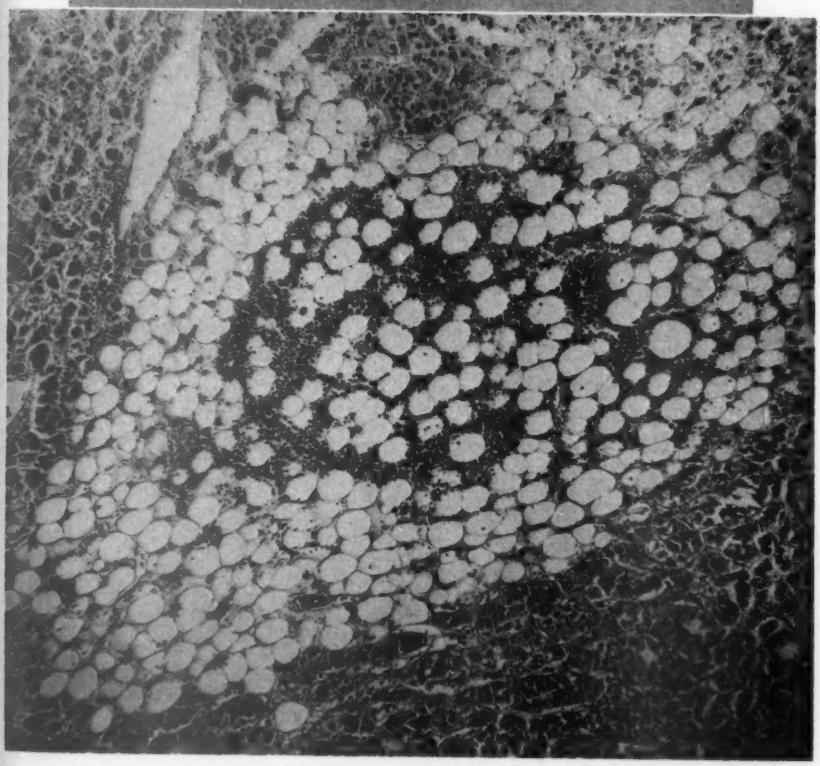

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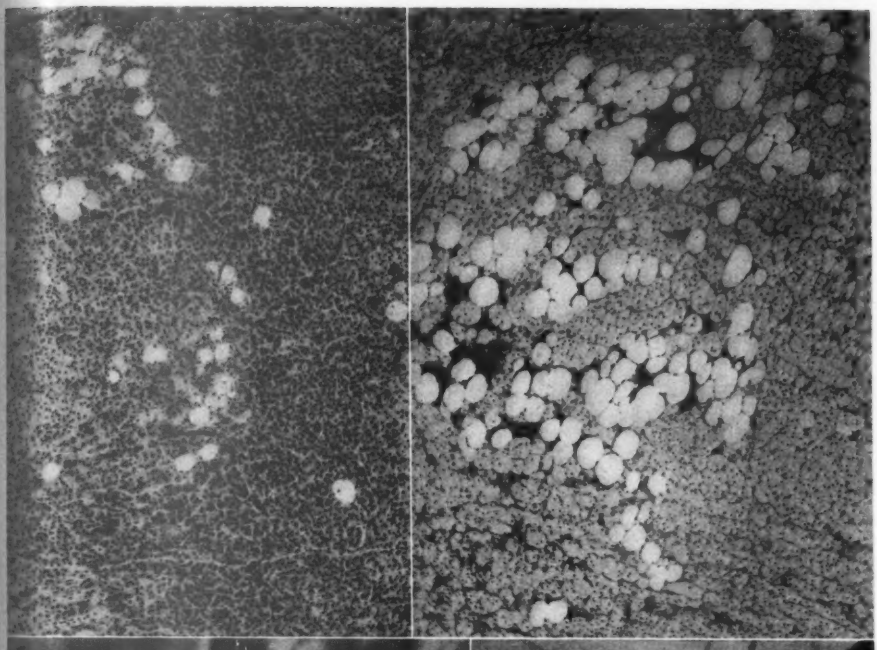
FIG. 9. Scattered and grouped fat cells. × 55.

FIG. 10. AFIP Acc. #727046, Neg. 56-22519. A larger but still discontinuous aggregation of fat cells and "lymphocytes." × 40.

FIG. 11. AFIP Acc. #665617, Neg. 56-11881. A large single fat cell. It was the only one in a large area of normal cortex. A cortical cell has been compressed into a narrow crescent at one margin. Small cells resembling lymphocytes are situated at the opposite margin. The larger of the two dark nuclei in a sinusoid to the left of the fat cell is probably in a myeloid cell. × 650.

FIG. 12. AFIP Acc. #640890, Neg. 56-9154. The diagonal line corresponds to the capsule of the adrenal which separates a myelolipoma from periadrenal fat. The curved row of 5 cells represents a remnant of cortex. Such patterns, especially when complicated by hemorrhage, are difficult to interpret. × 235.



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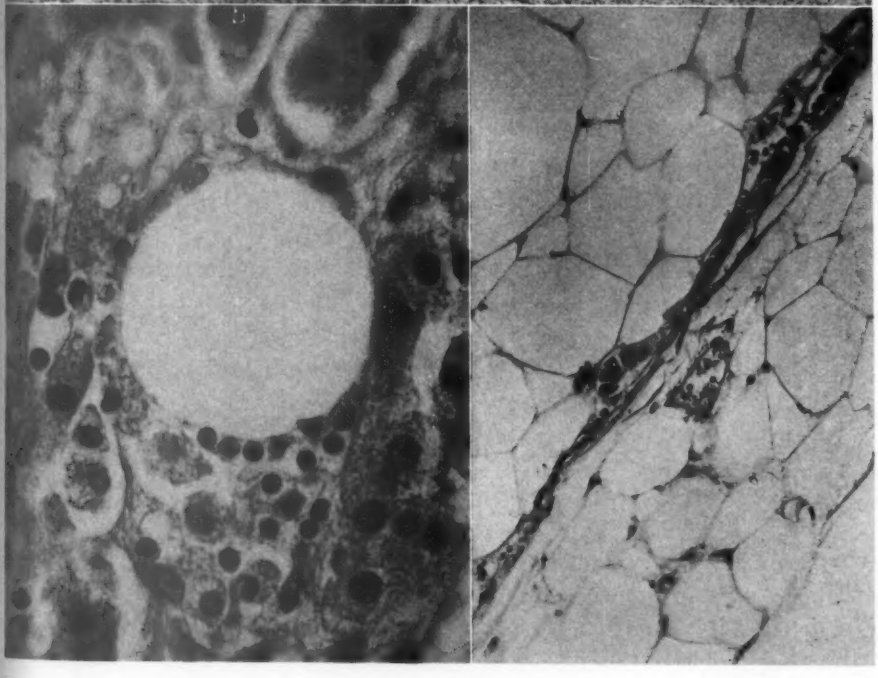

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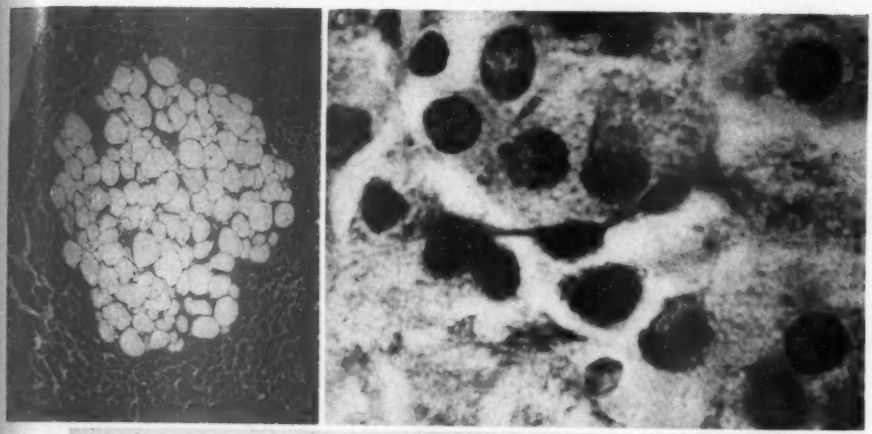
FIG. 13. AFIP Acc. #489929, Neg. 56-23604. A fairly well circumscribed accumulation of closely packed fat cells, perhaps a small myelolipoma. × 42.

FIG. 14. AFIP Neg. 56-5698. Myeloid cells in lumen of an adrenal cortical sinusoid of a rabbit. Note the swelling and intense staining of one sinusoidal lining cell. × 1,300.

FIG. 15. AFIP Acc. #360763, Neg. 55-7732. A centrally located myelolipoma. The round portion on the left is almost entirely fatty. The crescent-shaped darker area on the right contains degenerating cortical cells and myeloid cells. × 4.

FIG. 16. AFIP Acc. #647466, Neg. 55-9923. The large cells, some confluent, containing multiple fat droplets, have large, dark-staining nuclei which are not flattened against the periphery. These represent degenerating cortical cells. × 430.



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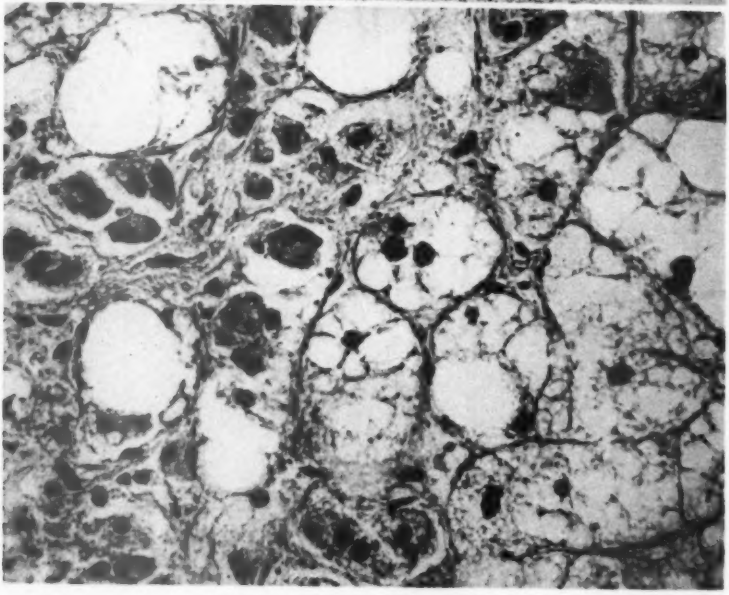


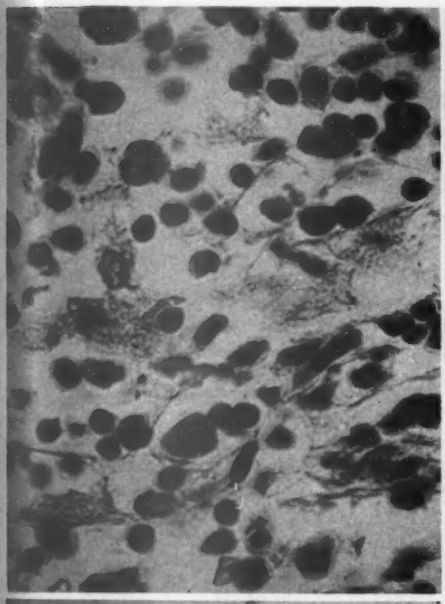
16

FIG. 17. Autopsy #29, 1951, V. A. Hospital, Topeka. AFIP Neg. 55-7251. While most of the cells resemble "lymphocytes," two are large and have irregular pyknotic nuclei, probably in prophase. They are best interpreted as myeloid elements. × 750.

FIG. 18. AFIP Acc. #649108, Neg. 56-17507. Megakaryocytes in a sinusoid of an adrenal cortical adenoma of a 14 year old dog. There were also other myeloid cells, but the cortex itself contained none. × 550.

FIG. 19. AFIP Acc. #611716, Neg. 55-8687. Bizarre, large nuclei in two degenerating cortical cells which are surrounded by fat cells. × 540.

FIG. 20. AFIP Acc. #560763, Neg. 55-7907. The broad, oblique band with the dark nuclei consists of cortical cells. At the upper right are characteristic fat cells, and at the lower left large granular cortical cells with remnants of nuclei. Such fields give the incorrect impression that cortical cells are transformed into fat cells. × 260.



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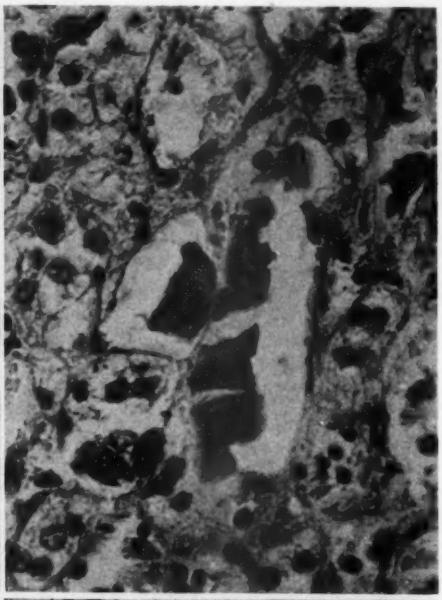

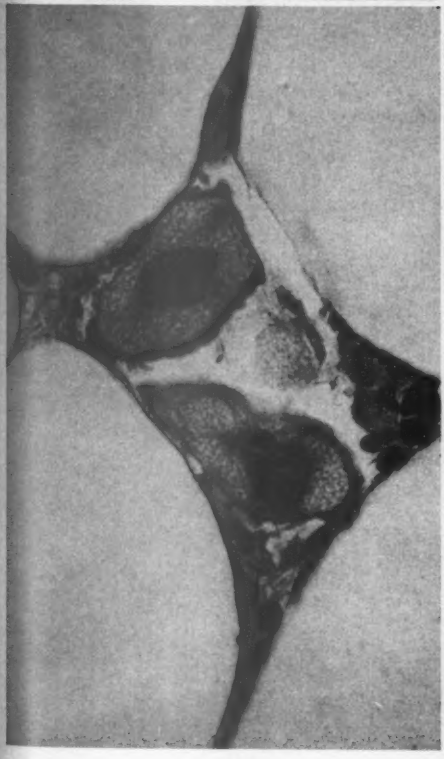

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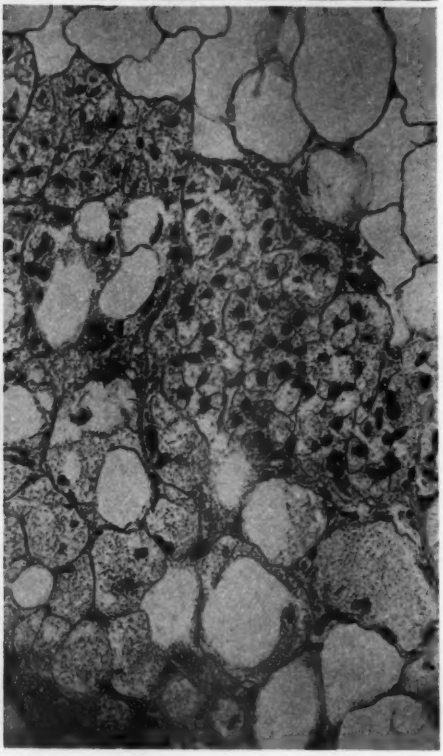
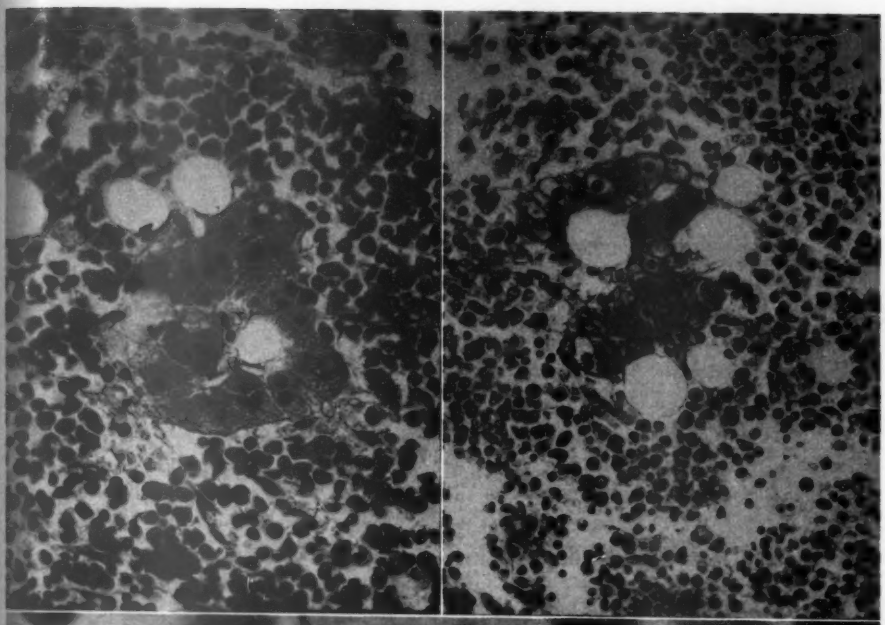


FIG. 21. AFIP Acc. #196568, Neg. 55-20568. A small island of well preserved cortical cells surrounded by myeloid elements of a myelolipoma. × 280.

FIG. 22. AFIP Acc. #196568, Neg. 55-20567. From a neighboring section. Wilder's stain for reticulum. The reticulum in the small island of intact cortical tissue is preserved but is missing in the surrounding myeloid area. × 280.

FIG. 23. AFIP Acc. #582504, Neg. 5154-1252. An area in an ordinary so-called cellular focus in adrenal cortex. The cells vary greatly in appearance and the designation "lymphocytes" does not seem appropriate. It is not possible to put a definite name on each cell. Some resemble plasma cells. × 1,700.



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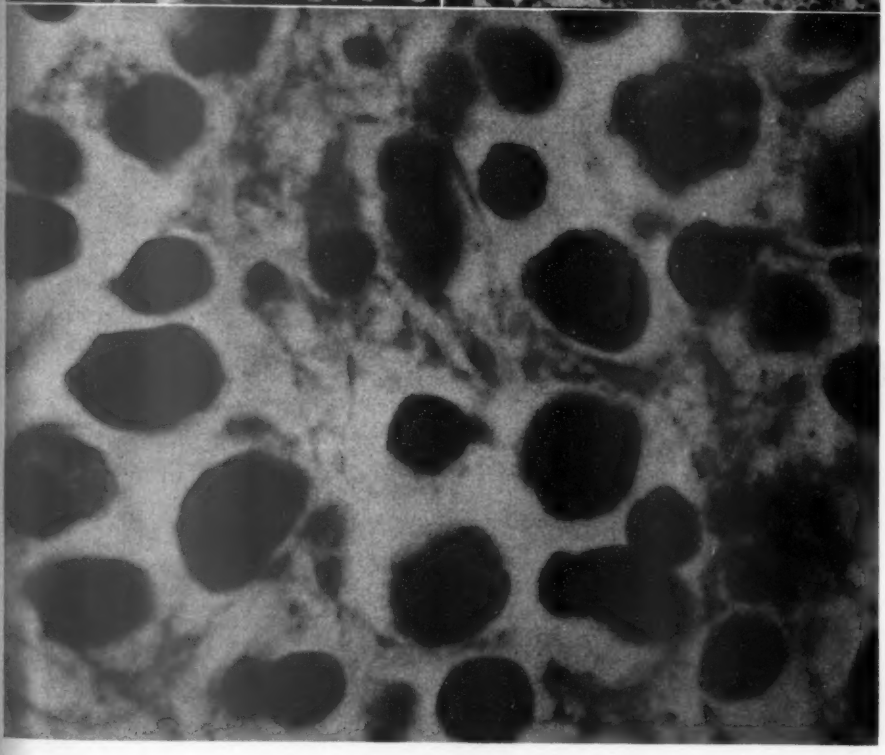

23

HISTOLOGY OF EXPERIMENTAL MURINE CRYPTOCOCCOSIS*

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This report is an outgrowth of a study in which strains of *Cryptococcus neoformans* were isolated from a number of pigeon nests in the Greater Cincinnati (Ohio) area.[1] The investigation followed that of Emmons[2] who reported the isolation of Cryptococcus from pigeon nests in Washington, D.C. In our own survey,[1] material was collected from 107 pigeon nests in order to document the concept that this organism had a wide geographic distribution. In 41 of the nests Cryptococcus was demonstrated by animal inoculation and culture. In the present paper we will describe the histologic changes produced in experimental animals after inoculation with material containing Cryptococci.

Material and Methods

The bird droppings procured from the pigeon nests were suspended in a mixture of antibiotic agents and saline solution (2,000 units each of penicillin and streptomycin per ml.) and introduced intraperitoneally into mice. The organs of each animal which died spontaneously or was sacrificed after 5 weeks were used for cultures on Sabouraud's glucose agar at 25° C. As indicated above, strains of *Cryptococcus neoformans* were isolated from 41 nests.

Male mice were inoculated with 3-day-old cultures of these Cryptococcus strains intraperitoneally (0.5 ml.) and intracerebrally (0.05 ml.). The suspension was uniformly heavy, but no actual cell counts were made. The animals began to die 6 days after inoculation, and surviving animals were sacrificed after approximately 5 weeks. Cultures were made from the liver and spleen of each animal. Specimens of liver, spleen, brain, lung, heart, and pancreas were fixed in 10 per cent formalin solution and sectioned in paraffin. In addition to this material, sections of these organs from the 367 mice which had been inoculated directly with pigeon dropping suspension were also studied. The sections were stained with hematoxylin and eosin, mucicarmine,[3] and by Grocott's[4] and Gridley's methods.[5]

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Observations

A great variation was observed in morbidity and mortality of experimental animals, and no clear-cut pattern of response was established. More animals died following intracerebral than after intraperitoneal inoculation, and the organisms were more often demonstrable in the brain and lung in the former group. At necropsy some animals exhibited hepatosplenomegaly, but this feature was not indicative of

Table I

Description of Source of Tissue Used in This Study

Material injected	Route	Number of animals		
		Injected	Dead (spontaneously)	Sacrificed
Suspension from pigeon nests	Intraperitoneal	367	162	205
Culture suspension	Intracerebral	46	29	17
Culture suspension	Intraperitoneal	46	11	35

infection with the Cryptococcus, since the cultures from some livers and spleens were negative. Furthermore, the yeast cells were not always demonstrated in sections of these organs even when inflammatory lesions were present, or when Cryptococcus appeared in the cultures. For convenience in this histologic analysis, the lesions as encountered in each organ will be enumerated. Since our purpose is to describe the lesions, there appears to be no need to indicate which were produced by direct inoculation of nest material or which by inoculation of culture suspension. In the direct suspension group, the lung, liver, spleen, and retroperitoneal tissue were more often the seat of lesions whereas in the animals inoculated with culture suspension there was also a marked involvement of brain, leptomeninges and fewer lesions in the pancreas.

The colonies of Cryptococci in culture showed variation in color and texture. This was the case even in instances in which they were isolated from the same pigeon nest. In tissue sections, however, the yeast cells were structurally indistinguishable from each other. Their average diameter without the capsules was 6.5 μ and with the capsule 17 μ. Most yeast cells were round or only slightly ovoid and had a variable width of capsule surrounding each organism. Budding was occasionally seen. In general, the appearance of the yeast cells was identical with that seen in human torulosis.[6]

Examination of Tissue Sections

Lungs. These consistently revealed the most severe inflammatory responses to the yeast cells. One type of lesion was characterized by diffuse inflammation and another by localized granuloma formation consisting for the most part of histiocytic elements, accompanied by occasional epithelioid cells and rare giant cells of the Langhans type. The presence of organisms in the alveoli or in the interstitial tissue without any noticeable reaction was also observed. The large number of yeast cells noted in some instances was striking, and the tissue alterations were thought to be the result of simple mechanical pressure rather than destructive inflammation. The thick capsules of the Cryptococci caused marked distention of alveoli with rupture of the interalveolar septa and pressure atrophy of adjacent tissues. In certain instances this culminated in the production of discrete areas of necrosis.

Diffuse inflammatory reaction was manifested by hyperemia and the accumulation of neutrophils in the lumens of the affected capillaries. Congestion was occasionally quite pronounced and was associated with alveolar edema. The edema fluid contained varying numbers of leukocytes. The exudate ranged from a scanty infiltration with widely distributed leukocytes to cellular aggregates gathered in perivascular and peribronchial tissues. Occasionally there were purulent bronchitis, peribronchitis, bronchopneumonia, and multiple minute abscesses. In addition to neutrophils there was almost always an intermixture of mononuclear cells (Fig. 1). Microscopic foci of atelectasis were induced by the widening of interalveolar septa containing yeast cells and inflammatory elements. The desquamation of alveolar lining cells mimicked a cellular inflammatory exudate, especially if with these elements there were also yeast cells.

The other common form of response was characterized by a diffuse or discrete granulomatous reaction in the pulmonary parenchyma. Yeast cells were readily recognized in the granulomas which were initially characterized by focal histiocytic proliferation. The yeast cells either remained free or were engulfed by macrophages. Present also were multinucleated foreign body giant cells (Fig. 2). The granulomatous lesions appeared around the blood vessels and bronchi. Necrosis in the center of the lesions was not observed (Fig. 3). The granulomas were characteristically discrete but exhibited a peripheral zone containing lymphocytes and scattered histiocytes. Neither fibrous encapsulation nor major scarring were encountered during the period of

investigation. Rarely, the lungs were so heavily studded with granulomas, and the component histiocytes so closely resembled epithelioid cells that the pattern of sarcoidosis was simulated. A disproportionately large number of yeast cells appeared in those lungs which showed minimal or no inflammatory response. On the other hand, the number of Cryptococci in the granulomas was always small, and in a few cases no organisms were demonstrable microscopically at all.

Liver. The incidence of hepatic lesions was exceeded only by that of the lung and the brain. As in the lung, there was a range of alteration from the presence of organisms without related inflammation to the development of granulomas. The encapsulated organisms singly or in clusters often caused pressure atrophy of adjacent tissue or distortion of liver architecture. The large, almost empty cavities thus produced, unaccompanied by an inflammatory reaction, caused a "Swiss cheese"-like appearance. In the presence of smaller clusters of Cryptococci, contiguous liver cells were flattened and stretched about the mass of yeast cells, simulating thin capsule formation.

In addition to the features described, the liver exhibited congestion and an acute or chronic inflammatory exudate, often with perivascular location. The cellular reaction, when restricted to the vicinity of a vessel, maintained a peculiar circumscribed appearance which resembled a tubercle and, indeed, was occasionally composed of epithelioid and giant cells. Not all of the lesions contained fungi, and when yeast forms were present, the exudate was often quite sparse. Granulomas were observed more often following inoculation of culture suspension intraperitoneally than following inoculation of pigeon dung suspension. The phagocytic activity of the macrophages was essentially identical with that observed in the lung.

Spleen. Clear cut splenic lesions were observed only in animals inoculated with culture suspension. The introduction of the pigeon nest preparation resulted in the appearance of a few solitary Cryptococci in the organ without obvious reaction apart from hyperemia. With the culture suspension the response varied from hyperemia to granuloma formation. In a few cases there were circumscribed foci of necrosis in which no yeast cells were recognized. Occasionally, the spleen was so heavily studded with yeast cells that the architecture was obscured, and though the pattern was that of an overwhelming infection, there was almost no inflammatory reaction. In those instances in which inflammatory cell infiltration and granuloma formation occurred, epithelioid cells and giant cells were evident. They appeared, in the main, in the splenic hilar area and extended to adjacent peritoneal tissue (Fig. 4).

Pancreas. This organ revealed less alteration than the liver or spleen. The lesion consisted of either a diffuse inflammatory cellular process or a perivascular microgranuloma formation. In addition, groups of Cryptococci were collected in tissue spaces unaccompanied by inflammatory response. The neutrophil or mononuclear infiltration was often localized to the surface of the pancreas or appeared in the peripancreatic tissues. In a few cases, small foci of necrosis were observed within the parenchyma.

Heart. The heart exhibited no inflammatory reaction. Scattered solitary fungi or small clusters of yeast cells appeared in the interstitial tissue or were embedded directly in muscle fibers. The presence of the large organisms caused pressure atrophy and was manifested in tissue sections by the appearance of small, sharply outlined holes (Fig. 5).

Meninges and Brain. With the exception of the lung, the meninges and the brain were the most frequent sites of involvement. The fungi were usually easily identified with the mucicarmine stain and were found either clustered focally or distributed fairly evenly in the leptomeninges (Fig. 6). The inflammatory response in the meninges was characterized in some cases by hyperemia or edema and in others by a cellular infiltration with polymorphonuclear leukocytes, lymphocytes, or monocytes. An exudate consisting entirely of lymphocytes was more often encountered (Fig. 7). The fungi were found lying free or in macrophages. The tissue response in some cases was granulomatous, and in others was of "meningothelial" nature. The "meningothelial" reaction was characterized by diffusely distributed lymphocytes producing a very cellular appearance in the subarachnoid region. In such cases the quantity of demonstrable fungi was small. In those with granulomatous lesions, neutrophils and lymphocytes were scanty.

In each instance the brain contained numerous fungi, but the cellular response to these was variable. The presence of free fungi in the lumens of blood vessels was reasonable indication that the brain lesions were of embolic nature (Fig. 8). Conglomerate masses of fungi were found in both the gray and white brain substance. Capsule development was often so pronounced that large holes were created with resemblance to "Swiss cheese" (Fig. 9). In these cysts fungi were quite obvious even without special stains. The multiplication of yeast cells created pressure effects on brain tissue which were rarely associated with cellular exudation. However, this was not always the case, and in many sections discrete round cell collections or nodular microglial reactions were observed. In some instances direct extension from the meninges was associated with lesions in the cortex along the perforating vessels. This form of propagation was particularly well demonstrated if the

plane of section happened to be in the axis of the vessel. One could then observe a perivascular cellular exudate consisting predominantly of either neutrophils or lymphocytes accompanied by smaller numbers of histiocytes. Small leukocytic conglomerations resembling microabscesses were also observed. The yeast cells were easily recognized in the perivascular spaces, where they appeared singly or in aggregates. The latter often led to cyst formation. In sections stained with hematoxylin and eosin, the paradoxical pattern of empty holes crossed by denuded vessels was noted. Mucicarmine stains, however, always demonstrated the presence of fungi in these cavities.

Discussion

The cellular response to *Cryptococcus neoformans* is nonspecific, variable and pleomorphic. Much of the variation probably reflects the histologic nature of the organ concerned. One never can be sure, however, whether the reaction in a given period will be of exudative or granulomatous character. Although the yeast cells can be demonstrated readily in the majority of cases, isolated lesions may be recognized when the organisms are not detected.

The pathologic lesions of experimental cryptococcosis have been analyzed by Levine, Zimmerman and Scorza,[7] who have provided a detailed description of the histologic features, especially in the skin and brain. The organisms used in their studies were isolated from human cases of cryptococcosis. While they established the existence of relatively prompt inflammatory reaction in certain organs and a delayed reaction in the brain, they also noted a relative immunity of striated muscle in spite of heavy inflammation in adjacent tissues. This to some degree parallels our own observations in the myocardium. Littman and Zimmerman[6] in an admirable monograph discussed the problem of cryptococcosis in detail from both the laboratory and clinical aspects. However, they did not discuss the histologic lesions in experimentally infected animals very extensively.

Summary

The histologic lesions in experimental animals inoculated with *Cryptococcus neoformans* have been described. The strains of Cryptococcus used in this experiment were isolated from pigeon nests in the Cincinnati area. Brain and lungs were the most frequent sites of lesions. The histologic pattern was found to vary from organ to organ even in the same animal. The heart muscle failed to respond with inflammation to the invasion by yeast cells.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Pneumonitis following inoculation with Cryptococcus (strain 38). Some yeast cells lie within the cytoplasm of macrophages. Mouse, spontaneous death on seventh day. Hematoxylin and eosin stain. × 500.

FIG. 2. Inflammatory giant cell response in lung of mouse inoculated intraperitoneally with Cryptococcus (strain 38). Hematoxylin and eosin stain. × 500.

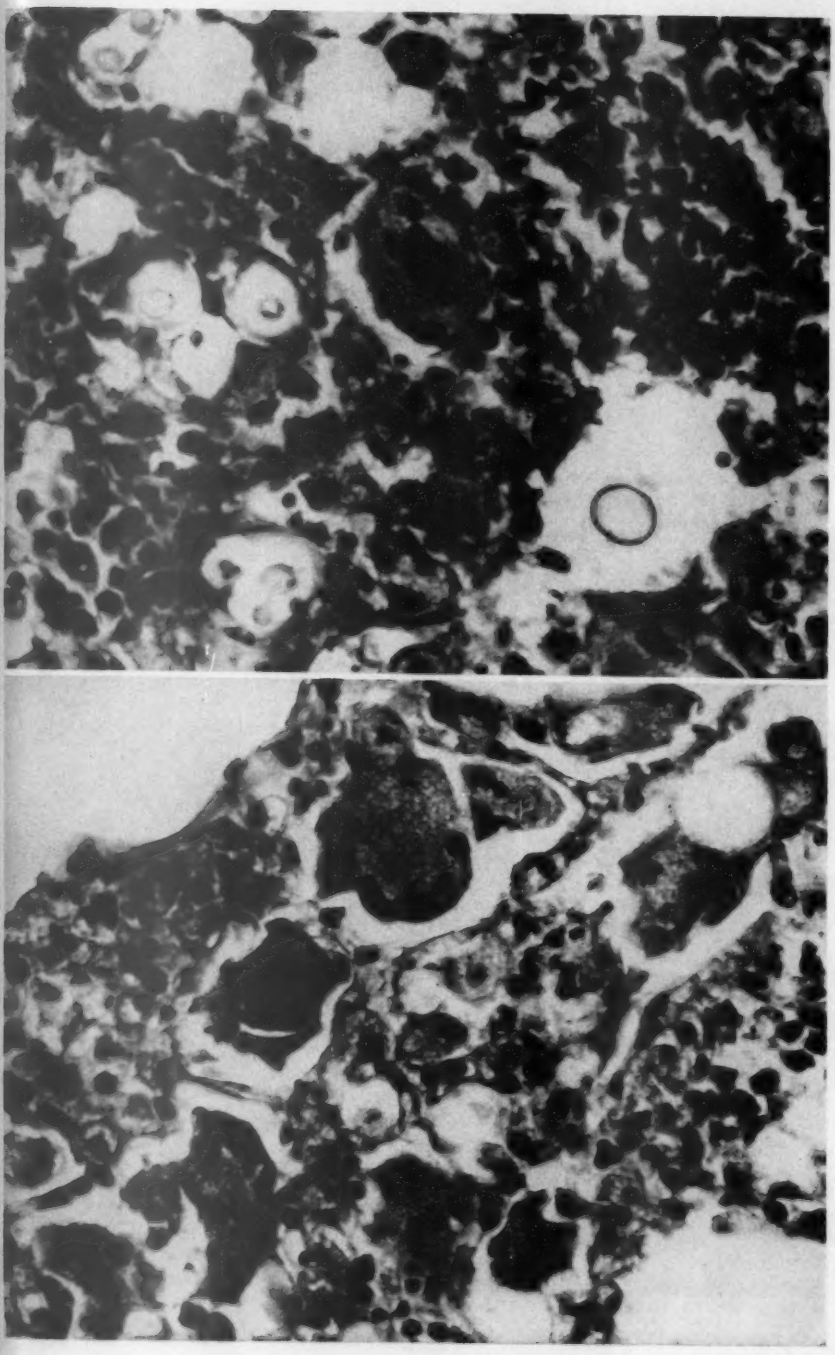

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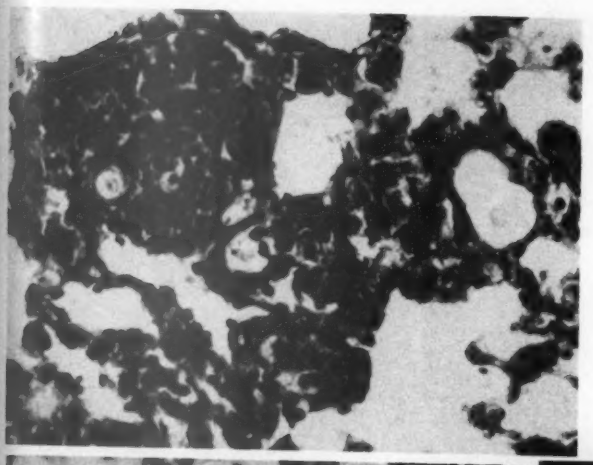
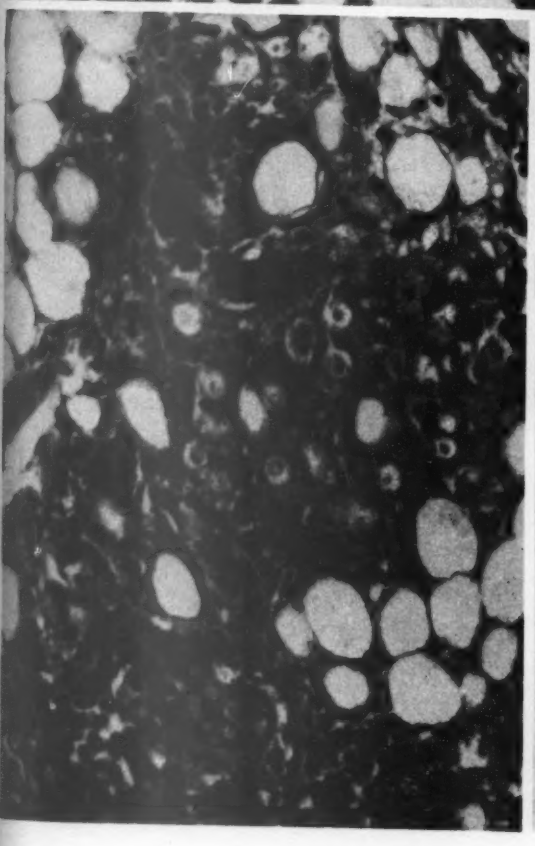
FIG. 3. Microgranulomatous lesion in mouse lung, obviously caused by Cryptococcus (strain 69). Inoculation with a suspension from a pigeon nest. Hematoxylin and eosin stain. × 250.

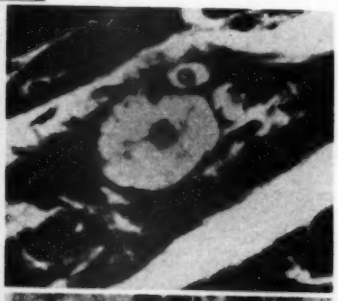
FIG. 4. The splenic hilus consistently shows inflammation after intraperitoneal inoculation. (strain 52.) Hematoxylin and eosin stain. × 250.

FIG. 5. Heart of a mouse inoculated intraperitoneally, death on 23rd day (strain 9). The yeast cells are lodged in heart muscle and interstitial tissue without manifest cellular reaction. Hematoxylin and eosin stain. × 500.

FIG. 6. Diffuse distribution of yeast cells in meninges 30 days after intraperitoneal inoculation with Cryptococcus (strain 48). Mucicarmine stain. × 500.







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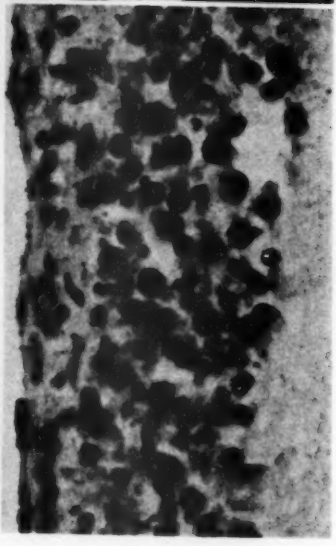

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FIG. 7. Cryptococcal meningitis with exudate dipping into a sulcus, 9 days after intracerebral inoculation (strain 51). Hematoxylin and eosin stain. × 250.

FIG. 8. Cryptococcus yeast cell "in transit" in a pulmonary vessel, 30 days after intracerebral inoculation (strain 54). Hematoxylin and eosin stain. × 1,000.

FIG. 9. Aggregation of Cryptococci in subcortical cerebral area without reaction in the immediate vicinity. "Swiss cheese" appearance. In contrast, note perivascular encephalitis in the same field (without organisms). Mouse, 80 days after intracerebral inoculation (strain 23). Hematoxylin and eosin stain. × 250.

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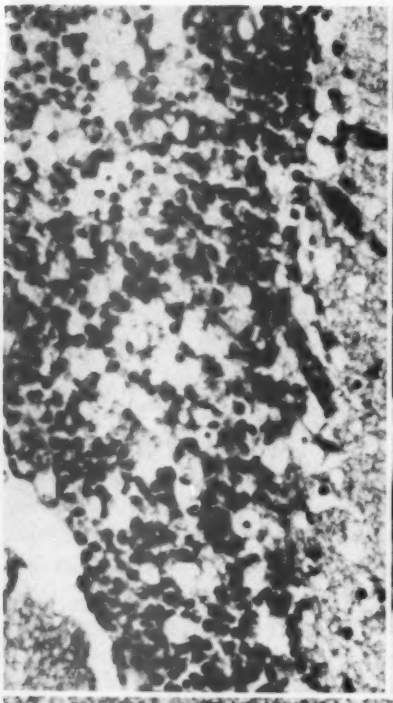

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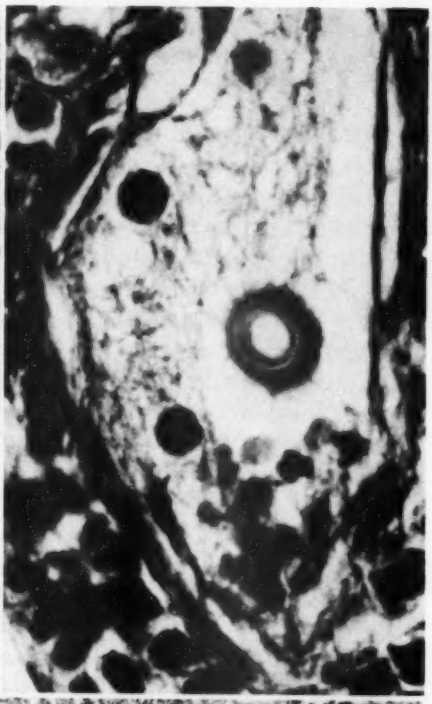

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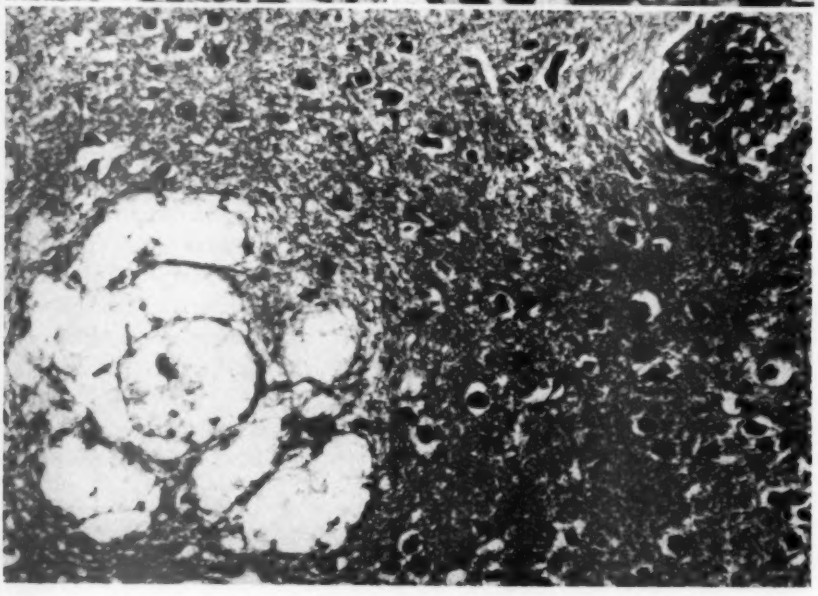

9

CARCINOMA OF THE STOMACH IN HIROSHIMA, JAPAN*

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More than 12 years have now passed since the atomic bomb exploded over Hiroshima. Concern about the possible effects of radiation on the well-being of the survivors is frequently expressed. Liebow, Warren and DeCoursey,[1] in their extensive report on atomic bomb casualties, remarked the need for a study of the relationship of radiation to a possible increase in the incidence of neoplasm as one of the major problems to be investigated in Hiroshima and Nagasaki. When the program of the Atomic Bomb Casualty Commission was initiated in 1947, the investigation of this matter was one of its objectives. At that time it was thought possible and, by some, even probable that the delayed effects of radiation would manifest themselves in striking increases in the incidence of a few specific diseases in the exposed populations. A rigid statistical control of the program was not instituted and, indeed, was impossible because of the varying degrees of cooperation offered to the Commission by the survivors. It was recognized that any difference in the incidence of a disease between exposed and nonexposed groups of patients would have to be quite pronounced in order to be detected and to withstand critical evaluation. Significant increase of the incidence of leukemia[2-5] and cataracts[6-8] in the exposed populations have been demonstrated.

In 1955 it was decided that the Pathology Department of the Atomic Bomb Casualty Commission should undertake an investigation of the occurrence of carcinoma of the stomach in both exposed and nonexposed individuals in Hiroshima. The project, as originally outlined, was to determine any differences in incidence, age of onset, or type of gastric tumor occurring in the population group exposed to mass irradiation as compared to a nonexposed population. Carcinoma of the stomach was selected because of its prominent incidence among neoplasms in Japan.

In an extensive survey of the "Geographical Pathology of Cancer in Japan,"[9] Takeda stated that cancer, in general, occurred less frequently in Japan than in Europe and in the United States. If, however, the death rate from carcinoma of the stomach were corrected according to the age distribution of the population, its incidence appeared to be

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higher in Japan than in western countries. Takeda stated that carcinoma of the stomach was the most frequent cancer in both men and women in Japan. He estimated that 43 per cent of all malignant neoplasms in Japanese men occurred in the stomach and that the corresponding figure for women was 30 per cent. The ratio was 1.25 afflicted males to one female in Takeda's series of carcinomas of the stomach; in the United States the ratio is about 1.5. Carcinoma of the stomach is third in frequency among neoplasms occurring in men and fourth in women and accounts for about 35 per cent of all cancer deaths in the United States.[10-15]

Materials and Methods

The Pathology Department at the Hiroshima Atomic Bomb Casualty Commission initiated full operation in December, 1948. The data listed at present in the diagnostic files have been collected by many Japanese and American pathologists who have been members of the department since that time. From December 1, 1948, through June 30, 1957, members of the department performed 880 necropsies upon adults. Of these, 464 were exposed patients and 416 were nonexposed. During the same period, 11,119 surgical biopsy specimens were examined; 3,185 were procured from exposed patients and 7,915 from nonexposed patients. In 19 cases the locality of the patient on August 6, 1945, could not be determined. In order for an individual to be classified as exposed to the atomic bomb, he must have been within 10,000 meters of the hypocenter when the bomb exploded. This figure was originally selected because one of the borders of Hiroshima City lies at that distance from the hypocenter.

In the necropsy series the principal cause of death was neoplastic disease. Of the 880 patients, 291 or 33 per cent, died as a result of a malignant neoplasm; 153 of these patients had been exposed and 138 had not been exposed to the explosion of the atomic bomb. About 68 per cent of the neoplasms observed in the series were carcinomas, and more than 35 per cent of these were located in the stomach. Carcinoma of the stomach was the most common neoplasm listed in the diagnostic file for both the necropsy and the surgical pathology series.

In undertaking the review of these cases, it was recognized from the outset that the total number of cases might not be large enough to be statistically significant, that the age distribution of the population in Hiroshima was constantly changing and was different for the exposed and nonexposed groups, and that certain other influences might have been introduced into the selection of the patients. The population of Hiroshima has been growing constantly since World War II, and at

present, approximately 90,000 survivors exist in a total population of 400,000. The average age of the survivors is of course somewhat older than that of the nonexposed group because the latter is, to a large extent made up of young newcomers to Hiroshima and children. Both the necropsy and surgical specimens examined at the Atomic Bomb Casualty Commission came from the Hiroshima hospitals. The Commission facilities maintain only 8 beds in internal medicine, and these are used for the study of interesting cases in cooperation with local physicians. No surgical procedures are carried out at the ABCC. Permission to do necropsies is obtained through a departmental necropsy contactor. Two times a day he contacts the City Hall and obtains reports of deaths in Hiroshima. He then approaches the next of kin, either through the attending physician or directly, and asks that the Atomic Bomb Casualty Commission be given permission to perform a necropsy. It is generally not possible to reach all of the families, and occasionally the report of death is delayed beyond the time when the performance of a necropsy would be useful. In the case of a nonexposed individual, if permission for necropsy is at first refused, no further effort is usually made. However, if the patient was among those exposed to the explosion of the bomb or if the attending physician is particularly interested in having a necropsy in either an exposed or nonexposed person, a further effort is made to obtain the consent of the family. Thus, the total number of exposed patients among the 880 necropsied cases was 464, a much higher proportion than would be expected by the ratio of exposed to nonexposed people in the population of Hiroshima. Approximately one fifth of the necropsies, whether on exposed or nonexposed patients, are performed at the direct request of the attending physician. In addition to our Autopsy Service, there are two hospitals in Hiroshima in which necropsies are performed. These hospitals occasionally concede the necropsy examination to the Atomic Bomb Casualty Commission or if the family agrees, invite a member of the department to attend the necropsy and examine the tissues.

The number of surgical pathology specimens examined in Hiroshima is small compared to those in an American city of comparable size. The great majority of specimens are examined at the Atomic Bomb Casualty Commission Pathology Department. Some specimens are received by mail from outlying districts. At the present time, the volume is approximately 2,500 specimens a year, but the number is now rising fairly sharply because of the establishment of a citywide Tumor Registry sponsored jointly by the Hiroshima City Medical Society and the ABCC Department of Pathology. This Registry began function-

ing on May 1, 1957. Our surgical pathology requisition forms and formalin specimen jars are made available and distributed to all of the hospitals and private physicians' offices in Hiroshima. A representative of our Contacting Service visits the different hospitals and other designated collecting points at least 3 times a week in order to pick up new surgical specimens and to return completed reports. No effort is made in the Surgical Pathology Service to emphasize particularly the collection of specimens from exposed patients. However, the information as to exposure is obtained on all patients and made a permanent part of the record. The great majority of the specimens come from gynecologic and general surgical sources. In some instances we have received surgical biopsy specimens from patients who subsequently are necropsied in our department. Thus, the present series of 535 patients with carcinoma of the stomach is represented in our files by a total of 562 surgical and necropsy records because necropsies were performed on 27 patients with carcinoma of the stomach who had been counted previously in the series by reason of surgical specimens. On the basis of reports from the 6 main hospitals and some of the larger private clinics in Hiroshima, it is estimated that about one sixth of the surgical specimens in Hiroshima are received for microscopic examination. Most of the remaining five sixths of the specimens are not examined histologically at the present time.

Data

The 535 patients in this series represent all the cases of carcinoma of the stomach recorded in our surgical or necropsy records from December 1, 1948, through June 30, 1957. All were confirmed by histologic study. There were 342 males and 193 females. This represents a male to female ratio of 1.77 to 1. The male to female ratio in the nonexposed patients was 1.8 to 1, and in the exposed patients 1.7 to 1. Of the total number of specimens, 461 were first examined as surgical specimens and 74 were first encountered at necropsy. In the Autopsy Service, a total of 101 carcinomas of the stomach were observed, but 27 of these had been included in the series by reason of earlier surgical specimens. Of the 461 specimens first seen in the Surgical Pathology Service, 132 were procured from exposed patients. Of the 74 cases in the necropsy group, 55 were exposed patients. Of the males, 224 were nonexposed control cases and 118 were exposed. Of the females, 124 were nonexposed and 69 were exposed.

The males in the series ranged in age from 22 years to 85 years, with an average age of 55.7 years. The females ranged from 23 years to 81 years, with an average age of 51 years. The average age of the non-

exposed males was 53.6 years and the nonexposed females, 48.4 years. The average age of the exposed males was 59.4 years and the exposed females, 52 years.

In 438 cases the description of the neoplasm was sufficiently complete to establish its location in the stomach. In the other cases either the description was incomplete or only a small fragment of the specimen was sent in by the surgeon with only the statement "tumor of the stomach." In two cases there were two separate carcinomas of the stomach. One was obtained from an exposed patient and had separate carcinomas on the posterior wall and on the greater curvature. The second was not exposed and had separate carcinomas in the pylorus and in the cardia. The distribution of the lesions according to the descriptions is indicated in Table I. The regions cited represent the apparent main location of the lesion. Most of the tumors along the lesser curvature also extended to either the anterior or posterior wall or both, and about 80 per cent of them extended into the prepyloric region.

TABLE I

Distribution of Gastric Carcinomas

Pylorus	66
Prepyloric region (including antrum)	107
Lesser curvature	185
Greater curvature	33
Limited to anterior wall	15
Limited to posterior wall	22
Fundus (anterior or posterior wall or curvature not specified)	6
Diffuse (*linitis plastica*)	4
Total	438

TABLE II

Histologic Pattern of Gastric Carcinomas

Adenocarcinoma	304
Mucinous adenocarcinoma	95
Undifferentiated carcinoma	102
Papillary adenocarcinoma	20
Linitis plastica—undifferentiated	9
Adenocanthoma	2
Unknown	3
Total	535

The histologic patterns were noted and the sections were reviewed (Table II). Although it was recognized that carcinomas of the stomach are all derived from glandular epithelium, it was possible to separate them into several histologic types. A previously published Japanese report[16] indicated that histologic differences between carcinomas of the stomach in exposed and nonexposed patients could be detected. We were unable to confirm this observation.

An attempt was made to establish the date of death or survival to the end of 1956 in all the patients from whom the 405 surgical specimens were procured. This inquiry was made through the local physician or hospital or by letter to the last known address of the family.

In 153 cases the follow-up was unsuccessful. In 159 cases the date of death was established, and in 93 instances the patient was found to be still alive in February, 1957. Of the 93 living patients, 28 had been exposed to the atomic bomb and 65 had not. The duration of survival is shown in Table III.

TABLE III
Duration of Survival, Living Patients

Duration of survival	Number of patients
More than 6 years	2
5 to 6 years	5
4 to 5 years	1
3 to 4 years	12
2 to 3 years	16
1 to 2 years	22
One year or less	35
Total	93

TABLE IV
Distance from Hypocenter, Patients with Gastric Carcinoma

Distance	Number of patients
Less than 1,000 M.	2
1,000 to 1,500 M.	31
1,500 to 2,000 M.	30
2,000 to 2,500 M.	38
2,500 to 10,000 M.	86
Total	187

All of the patients, of course, had had surgical treatment for the neoplasm. The usual therapy for carcinoma of the stomach as carried out in Japan is a subtotal resection of the stomach with a partial or total resection of the omentum and regional lymph nodes. Total gastrectomy is usually not done. As can be seen in Table III, the very low 5 year survival prevailing in western countries is also found in Japan.

In the patients who were operated on and who subsequently died, the postoperative survival time was investigated to determine if there was any significant difference between the exposed and control groups. In all of the necropsy cases and in 159 of the surgical cases, the date of death was known. In only 20 of the 74 necropsy cases had gastrectomy been performed. It was possible to calculate the postoperative survival time in 179 patients who had died. Of this total, survival ranged from 0 to 51 months with an average of 9.2 months. The number of patients in the exposed group was 99, and their average survival time was 9.2 months. The average survival time for 80 nonexposed patients was 9.16 months. It will be noted that there was no difference in the average survival time between the control and exposed patients.

Of the 187 exposed patients in this series, there was a history of acute radiation illness in 16 cases. In 81 cases there was a definite denial of acute radiation sickness, and in 90 cases a radiation illness history was not taken. A complete history of acute radiation illness was available only in those patients who had attended the ABCC Out-

patient Department during life or had been necropsied. In the patients from whom only surgical biopsy specimens were received, the complete questionnaire concerning acute radiation illness was usually not available. The criteria for establishing the existence of acute radiation illness was based upon a history of epilation, oropharyngeal lesions, bleeding gums and purpura, any or all of which must have occurred within the first 60 days after the explosion of the atomic bomb on August 6, 1945. The farthest distance from the hypocenter for any of the 16 patients was 2,300 meters. It has been noted that the majority of patients who had signs and symptoms of acute radiation illness were within 2,000 meters of the hypocenter at the time of exposure,[3,17] and among the 16 patients only 3 were located at a greater distance when the bomb exploded. Only 2 of the patients in the carcinoma of the stomach series were exposed at less than 1,000 meters. The closest was at a distance of 600 meters, and this patient had had definite signs and symptoms of acute radiation illness, including oropharyngeal lesions, bleeding gums, purpura and epilation. He was 34 years old at the time of the bombing. The other patient was exposed at 780 meters, and he had not had acute radiation illness. The second patient was 60 years old in 1945. It is apparently impossible to determine which of the patients exposed at 2,000 meters or less had had acute radiation illness. In spite of the fact that only 16 of the patients had had radiation illness, 101 patients had been exposed at distances under 2,500 meters (Table IV).

Of course, the importance given to the distance of an exposed individual from the hypocenter must be qualified by the amount of radiation actually received. This was affected by shielding and perhaps by individual biologic variation. LeRoy[18] and others[19] have pointed out that bone marrow depression, as evidenced by leukopenia, occurred even in the absence of other symptoms of the acute radiation syndrome. Thus, individuals may have suffered radiation illness without overt manifestations. In addition, many errors of exaggeration and omission may have entered into the histories, especially since they were obtained as long as 12 years after the event. However, the fact remains that there are wide variations in the effects of acute radiation as indicated by histories from patients exposed at distances of 2,500 meters or less.

The incidence of carcinoma of the stomach among the exposed and the nonexposed patients listed in the combined necropsy and surgical records was calculated. This was also done for all neoplasms found in these groups. The results, as seen in Tables V and VI, show that there was no significant variation between the exposed and nonexposed groups, either for the incidence of carcinoma of the stomach or for

that of all neoplasms. In making these calculations, 18 cases were subtracted from the total number of patients necropsied in the exposed group and 9 from the control group because these cases had already been entered in the series in the surgical pathology group.

On the chance that the patients exposed between 2,500 meters and 10,000 meters had received so little radiation that for the purposes of this study they should be considered nonexposed, the calculations were again made, defining the exposed group as only those patients who were within 2,500 meters of the hypocenter. The remainder of the exposed group was added to the controls. Once again, almost no difference of incidence was found in the two groups, either for carcinoma of the stomach or for the incidence of all neoplasms (Tables V and VI).

TABLE V

Incidence of Carcinoma of the Stomach in the Necropsy and Surgical Pathology Files, Compared with Distance From the Hypocenter

	Total patients exposed under 10,000 meters	Total patients not exposed	Total patients exposed under 2,500 meters	Patients exposed from 2,501 to 10,000 meters & all nonexposed patients
All necropsy cases, Hiroshima ABCC	464	416	306	574
All surgical pathology cases, Hiroshima ABCC	3,185	7,915	1,780	9,320
Total specimens	3,649 (3,631 patients)	8,331 (8,322 patients)	2,086 (2,074 patients)	9,894 (9,879 patients)
Patients with carcinoma of the stomach	187	348	101	434
Percentage, carcinoma of the stomach, necropsy and surgical cases	5.2%	4.2%	4.9%	4.4%

TABLE VI

Incidence of All Malignant Neoplasms in the Necropsy and Surgical Pathology Files, Compared with Distance From the Hypocenter

	In patients exposed under 10,000 meters	In patients not exposed	In patients exposed under 2,500 meters	In patients exposed from 2,501 to 10,000 meters & all nonexposed patients
Necropsy cases, Hiroshima ABCC	153	138	137	154
Surgical pathology cases, Hiroshima ABCC	519	1,576	274	1,821
Total malignant neoplasms	672	1,714	411	1,975
Total patients represented	3,631	8,322	2,074	9,879
Percentage of malignant neoplasms	18.5%	20.6%	19.8%	20.0%

The total exposed populations were estimated in 1954 to be 47,600 in the 0 to 2,500 meter distance and 50,500 in the 2,501 to 10,000

meter distance. These estimates were based on the Atomic Bomb Casualty Commission's 1949 Radiation Census and on the Japanese National Census of 1950. The incidence of carcinoma of the stomach and of all malignant neoplasms in both of these groups, as shown by material collected at the Hiroshima ABCC pathology department, is demonstrated in Table VII. The small differences in percentages seen here

TABLE VII

Carcinoma of the Stomach and All Malignant Neoplasms
Incidence in Two Exposed Populations

Distance from the hypocenter	Population	Cases observed at Hiroshima ABCC			
		Carcinoma of the stomach		All malignant neoplasms	
		No. of patients	Incidence	No. of patients	Incidence
0–2,500 meters	47,600	101	.21%	411	.86%
2,501–10,000 meters	50,500	86	.17%	261	.51%

are not considered significant. The incidence for the nonexposed group could not be calculated since this population is a fluid one. Moreover, in the necropsy service, special emphasis has always been placed on obtaining material from exposed cases.

The age distribution of the patients with carcinoma of the stomach was determined (Table VIII), and the exposed group was again di-

TABLE VIII

Necropsy and Surgical Records: Carcinoma of the Stomach

Age	Patients exposed to atomic bomb		Patients not exposed to atomic bomb
	Exposed at 2,500 meters or less	Exposed between 2,500 and 10,000 meters	
21 – 25	0	1	3
26 – 30	2	0	11 (3.2%)
31 – 35	2	2	14 (4.0%)
36 – 40	4	7 (8.1%)	30 (8.6%)
41 – 45	7 (6.9%)	7 (8.1%)	40 (11.5%)
46 – 50	12 (11.9%)	10 (11.6%)	52 (14.9%)
51 – 55	11 (10.9%)	14 (16.3%)	52 (14.9%)
56 – 60	14 (13.9%)	11 (12.8%)	58 (16.7%)
61 – 65	17 (16.8%)	12 (14.0%)	53 (15.2%)
66 – 70	14 (13.9%)	10 (11.6%)	24 (6.9%)
71 – 75	12 (11.9%)	8 (9.3%)	7 (2.0%)
76 – 80	5	3	3
81 – 85	1	1	1
Total	101	86	348

vided into two subgroups: those exposed at 2,500 meters or less and those exposed between 2,500 meters and 10,000 meters. Again there was no significant variation in the incidence among the 3 groups in any 5 year period.

A preliminary analysis of the surgical pathology records indicated that carcinoma of the stomach occurred about 8 times more frequently among men than among women, whether or not exposed. The necropsy material was then examined and showed no significant difference in incidence between males and females. The discrepancy in the surgical pathology records is readily explained in view of the fact that the number of specimens from females is made very much larger by the submission of numerous cervical and breast biopsy specimens. In Japan, even more so than in the United States, these are not equated in the male series. The almost complete absence of prostatic hypertrophy or prostatic carcinoma in Japan[20,21] heightens the difference in numbers of surgical pathology specimens received from men and women.

The data gathered in this study were submitted to the Biostatistics Department in the hope that a real incidence of carcinoma of the stomach per 10,000 people could be calculated for the exposed and nonexposed groups. During the past 18 months, in an effort to give statistical significance to the work carried out at ABCC, the establishment of fixed population samples in Hiroshima for those exposed to the bomb detonation at close distances, those at greater distances, and those not exposed has been under way. The population sample for the nonexposed group is still incomplete, and therefore, it was not possible to calculate a real incidence of carcinoma of the stomach for this group. However, it was found that 42 carcinomas of the stomach from the group exposed under 2,500 meters, and 30 from the group exposed between 2,500 and 10,000 meters were included in the master sample. The population figures for these two groups are now available for the years 1951 through 1956. For these two groups, a valid incidence of carcinoma of the stomach per 10,000 live population could be calculated. This incidence is 1.80 per 10,000 for the group exposed under 2,500 meters and 1.72 per 10,000 for those exposed 2,500 to 10,000 meters. This, of course, constitutes no significant difference.

Comment and Summary

Carcinoma of the stomach was listed in 535 cases in the surgical pathology and necropsy pathology records of the Hiroshima Atomic Bomb Casualty Commission between December 1, 1948, and June 30, 1957. Of these patients, 187 had been exposed to the explosion of the atomic bomb in Hiroshima. The cases in the exposed group were com-

pared with those in the nonexposed group, and no significant differences were found between these two groups for the average age at the time of diagnosis, the age distribution in 5 year age groups, the postoperative survival time, or the histologic pattern and location of the tumor in the stomach. The incidence of all neoplasms in the exposed and nonexposed groups was also calculated and found to be almost equal. The possibility that the definition of "exposure" was too broad and was thus hiding effects in the patients exposed at 2,500 meters or less was considered, and the material was re-examined using 2,500 meters from the hypocenter as the limit of exposure. The patients between 2,500 and 10,000 meters were added to the control group. The incidences of carcinoma of the stomach and of all neoplasms were again calculated and again found to be approximately equal. In the cases treated by a surgical resection of the cancer through 1956, a follow-up was possible in 252 patients; 93 of these were still living, but only 7 had lived more than 5 years after the operation. Only one of these was an exposed patient. No significance was attached to this finding.

Carcinoma of the stomach is the most frequent neoplastic disease for both men and women in Japan. Its incidence and behavior in patients exposed and not exposed to the explosion of the atomic bomb in Hiroshima has been compared. No significant differences were found in these two groups. The study represents an 8½ year survey ending almost 12 years after the atomic bombing of Hiroshima.

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FIFTY-FIFTH ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

CLEVELAND, OHIO

APRIL 24TH, 25TH, AND 26TH, 1958

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Fifty-fifth Annual Meeting

HOTEL STATLER

Cleveland, Ohio

April 24th, 25th, and 26th, 1958

PRESIDENT FARBER IN THE CHAIR

BUSINESS MEETING

April 24, 1958

The following nominations for elective officers were submitted by the Council:

President	DR. ALAN R. MORITZ
Vice-President	DR. DOUGLAS H. SPRUNT
Secretary	DR. RUSSELL L. HOLMAN
Treasurer	BRIG. GEN. ELBERT DECOURSEY
Incoming Member of Council	DR. SIDNEY C. MADDEN

Additional nominations were called for. None having been offered, it was moved and seconded from the floor that the Secretary be instructed to cast a unanimous ballot for the entire slate.

At the direction of the President, the Secretary reported the following actions of the Council:

Election of New Members

William J. Beckfield	William J. Kuhns
John W. Berg	Sydney S. Lazarus
William A. Blanc	Harold Lepow
Robert P. Bolande	George D. Lumb
Slater M. Dozier	Arthur J. McAdams, Jr.
John H. Edgcomb	Joseph F. Metzger
Herbert Fanger	Richard D. Moore
Benjamin S. Gordon	Vincent Moragues
James H. Graham	Henry Z. Movat
John G. Gruhn	Rudolph J. Muelling, Jr.
James C. Harkin	Charles S. Petty
John Higginson	Anthony V. Postoloff
Robert B. Jennings	Floyd R. Skelton

Jack P. Strong	Lee W. Wattenberg
Frank M. Townsend	Richard B. Williams, Jr.
Jacinto J. Vazquez	Sumner Wood, Jr.

Election of Members of Editorial Board and Assistants to Officers

Assistant Secretary	Dr. Jack P. Strong
Assistant Treasurer	Dr. Elson B. Helwig
Editorial Assistant	Miss Janet E. Smith

Members of the Editorial Board

Dr. Hugh G. Grady . . .	Term to expire December 1962
Dr. Orville T. Bailey . . .	Term to expire December 1963
Dr. Howard T. Karsner . .	Term to expire December 1964

With deep regret, the recording of the deaths of:

William H. Bauer	D. L. Harris
Alfred Cohn	Philip Hartz
John Funke	Fritz Levy
Alfred Giordano	Mark E. Maun
John W. Hall	Charles R. Rein

Eugene Whitmore

The question of resignations and retirements was postponed pending legislative action.

The following amendment to the bylaws was read at the Business Meeting last year:

By Law 6 (dealing with method of amendment) was to become By Law 7.

By Law 6 (new)

(a) Members in good standing who by reason of age or physical disability have retired from gainful professional activity, may upon application to Council, be granted emeritus membership.

(b) Emeritus members shall remain upon the rolls of the Association and shall receive regular notices. They shall, however, be relieved of payment of dues and may neither hold office nor receive *The American Journal of Pathology* except by independent subscription.

These bylaws were passed by unanimous decision.

A new amendment to be read and voted upon at the next business meeting to become By Law 6, Section A:

"Members in good standing who have attained age 65 or who have retired from gainful professional activity because of physical dis-

ability may, upon application to Council, be granted emeritus membership."

The Secretary announced that the next Annual Meeting will be held in Boston, Massachusetts, April 23, 24, and 25, 1959. The topic for the symposium will be "Conditioning Factors in Neoplasia." The referee will be Dr. Jacob Furth.

The Secretary further announced that the Annual Meeting for 1960 will be held in Memphis, Tennessee, April 28, 29, and 30. The topic for the symposium will be "Disorders of Genetic Origin."

The decisions of Council regarding the type of program to be initiated this coming year in Boston are:

1. Program Committee to consist of President (Chairman), Vice-President, Secretary and Editor.

2. Announcement of meeting to be circulated early (approximately December 1), and the date of receipt of abstracts will be approximately January 15.

3. Number of papers selected for oral presentation will be fewer and will permit single sessions.

4. Time for papers will not exceed 15 minutes, except at the discretion of the Program Committee. Discussion will be encouraged and ample opportunity will be provided.

5. Abstracts of papers "Read by Title" will continue to be published in the *Proceedings*.

The President then asked if there was any business from the floor. None was presented, and the Business Meeting adjourned at 2:28 P.M.

Russell L. Holman, *Secretary*

REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted. It was accompanied by a letter of certification from Ralph Cole, Certified Public Accountant, of Washington, D.C. In condensed form, the Treasurer's report follows:

General Checking Account

Receipts

Balance on hand, January 1, 1957		$ 7,685.28
U.S. Government Series G bonds cashed	$20,000.00	
Membership dues	9,230.00	
Interest on bonds and savings accounts and dividends on shares with building and loan associations	1,308.22	
		30,538.22
Total receipts		$38,223.50

Disbursements

American Journal of Pathology	$ 7,396.00		
Equipping editorial offices	1,817.55		
		$ 9,213.55	
Secretary's office, clerical	$ 250.00		
Travel expenses	221.93		
Printing, supplies, miscellaneous	600.24		
		1,072.17	
Treasurer's office, bonding and auditing	$ 155.00		
Secretarial services 1956 and 1957	450.00		
Printing, supplies, miscellaneous	333.46		
		938.46	
Miscellaneous, annual meeting	$ 200.00		
Purchase of shares in building and loan association	15,000.00		
Deposit in savings account	5,000.00		
Refund	5.00		
National Society for Medical Research	50.00		
		20,255.00	
Total disbursements			$31,479.18
Balance on hand, December 31, 1957			$ 6,744.32

Investment Inventory

Savings accounts	
First and Citizens National Bank, Alexandria, Va.	$ 5,000.00
Riggs National Bank, Washington, D.C.	6,254.47
The First National Bank, Washington, D.C.	3,591.87
Home Savings & Loan Association, Los Angeles	5,000.00
Olympic Savings & Loan Association, Berwyn, Ill.	5,000.00
Atlantic Savings & Loan Association, Los Angeles	5,000.00
Mutual Savings & Loan Association, Pasadena, Calif.	5,000.00
Total of investment inventory	$34,846.34

Elbert DeCoursey, *Treasurer*

REPORT OF REPRESENTATIVE THE BIOLOGICAL STAIN COMMISSION

The Biological Stain Commission was created in 1922 under the auspices of the National Research Council by joint action of the major societies of chemists, botanists, anatomists, bacteriologists, and zoologists. The American Association of Pathologists and Bacteriologists was among the initial participants. Dr. Frank B. Mallory represented the Association from 1922 to 1940; Dr. Ralph D. Lillie has done so since 1940.

The Commission was created to establish and maintain standards in the production of dyestuffs for use in staining procedures. To this end the Commission applies certain chemical and spectroscopic tests in its own laboratory at Rochester, N.Y., and has each sample tested in a variety of specific staining techniques by cooperating members of the founding societies with pertinent competences.

The membership of the Association is urged to volunteer its assistance in testing samples and to suggest to the Commission new dyes for which certification should be required for specific purposes. This testing will serve to assure the reliability of the dye samples tested.

Currently the Commission provides compensation for technician time used in testing at $1.50 per hour. Those wishing to participate should communicate with Dr. Ralph D. Lillie or Dr. Victor E. Emmel, Secretary, Department of Anatomy, University of Rochester School of Medicine & Dentistry, Rochester, N.Y.

Ralph D. Lillie, *Representative*

SCIENTIFIC PROCEEDINGS

Abstracts

Lymphosarcoma in the Rat and C^{14}-labeled Bacterial Polysaccharide. Russell S. Jones,* University of Utah, College of Medicine, Salt Lake City, Utah.

The lymph nodes of the rat show the highest tissue concentration of C^{14} after the parenteral injection of a labeled polysaccharide complex derived from *Klebsiella pneumoniae*. The C^{14}-labeled, relatively nontoxic polysaccharide retains its haptenic properties after extraction from the lymph nodes.

As a study in the pathogenesis of the hemorrhagic necrosis of tumors induced by various polysaccharides, rats bearing Murphy lymphosarcoma were given a single intravenous injection of C^{14}-labeled *K. pneumoniae* polysaccharide, 1 mg. per hundred gm. body weight, and the tissue distribution of the polysaccharide determined by isotopic, extractive and immunochemical procedures. The tumor incorporated slightly less C^{14} per gm. than the cervical lymph nodes but far less than the mesenteric lymph nodes. Considerable isotope remained in the macrophages at the sites of the regressed tumor. After the first few hours the loss of polysaccharide from the plasma, liver and kidney was exponential, but the disappearance from the lymph nodes, spleen and adrenal was slower and more variable. Regression of the lymphosarcoma may be followed by redistribution of the polysaccharide. Tumor cells, implanted simultaneously with the injection of labeled polysaccharide, incorporated some C^{14} during their subsequent growth, apparently reflecting the low plasma levels of polysaccharide at this time. Besides the direct localization, plasma protein binding of polysaccharide, demonstrable by paper electrophoresis, may signify an important factor in the hemorrhagic necrosis of the tumor. The "stress" response induced by the injection of the polysaccharide does not appear significant since neither exogenous corticosterone nor cortisone affect the lymphosarcoma unfavorably.

Radioautographic Demonstration of Sulfur^{35} in Neoplastic and Certain Other Biopsied Tissues of a Patient with Chondrosarcoma. Richard L. Swarm, J. Robert Andrews, Philip Rubin, Lawrence Schlachter, Kirkland Brace, and Eliza Miller, National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

Following the demonstration in immature rats that the proliferating cartilage of the epiphysis is selectively destroyed by large doses of S^{35} given as inorganic sulfate, one curie of S^{35} as inorganic sulfate was administered in 3 divided doses to a patient with a well differentiated chondrosarcoma. Radioautographs were prepared from biopsy specimens of metastatic tumor, rib cartilage, skin, bone marrow and peripheral blood. Organically bound sulfate was found to be present in the intercellular matrix of the neoplasm and in normal rib cartilage. The concentration of bound S^{35} was greatest in the matrix immediately adjacent to apparently viable tumor cells. Little or no bound S^{35} was present in the intercellular matrix in areas of necrosis in the tumor. In this patient, the concentration of S^{35} was greater in the neoplasm than it was in the normal rib cartilage or skin. The presence of S^{35} in megakaryocytes and platelets was demonstrated in radioautographs of dried films of bone marrow and peripheral blood. This localization of S^{35} was associated with a temporary depression of platelets in the peripheral blood.

* Asterisks indicate members of The American Association of Pathologists and Bacteriologists. All others appear on the program "by invitation."

JUVENILE MELANOMAS OF CHILDREN AND ADULTS AND MELANOCARCINOMAS OF CHILDREN. Arthur C. Allen,* University of Miami School of Medicine, and Jackson Memorial Hospital, Miami, Fla.

The criteria for the histologic diagnosis of juvenile melanomas of children and adults are described and the clinical significance of these lesions is indicated. The histologic basis for the differentiation of juvenile melanomas from melanocarcinomas of children is outlined.

NECROPSY STUDY OF 257 PERSONS WITH CANCER AND 70 YEARS OF AGE AND OLDER. R. M. Mulligan,* University of Colorado School of Medicine, Denver, Colo.

Among 257 persons (194 men and 63 women) dying of cancer and necropsied at the Colorado General Hospital between 1940 and 1955, 199 were 70 to 79 years of age, 53 were 80 to 89, and 5 were 90 to 99. Since 3 men had 2 different lethal cancers, the total of lethal neoplasms was 260. Of these, 222 were carcinoma, 15 lymphosarcoma, 6 plasma cell myeloma, 5 myelogenous leukemia, 5 glioma, 3 malignant melanoma, and 1 each monocytic leukemia, malignant neurilemmoma, liposarcoma, and neuroblastoma. The primary sites of the neoplasms were as follows: stomach, 36; large intestine, 34; prostate, 33; hematopoietic system, 24; lung, 22; pancreas, 18; gallbladder, 15; urinary bladder, 11; skin, 8; breast, 6; bile duct, 5; brain, 5; kidney, 5; cervix, 4; esophagus, 4; ovary, 4; liver, 3; pharynx, 3; retroperitoneum, 3; thyroid, 3; eye, 2; small intestine, 2; larynx, 2; tongue, 2; and gingiva, lip, palate, penis, thymus, and vulva, 1 each. Among the 257 patients, 21 also showed a nonlethal carcinoma affecting the prostate in 11, the kidney in 3, the breast in 2, and the gallbladder, large intestine, larynx, pancreas, and tongue in 1 each. About 900 other persons 70 years of age and older, dying of other diseases in this 15-year period, showed 75 nonlethal cancers of which 71 were carcinoma, 2 were lymphosarcoma, 1 was a carcinoid tumor with metastasis to the liver, and 1 was a liposarcoma.

INTRAGLANDULAR DISTRIBUTION OF THYROID CARCINOMA AS SHOWN BY WHOLE ORGAN SUBSERIAL SECTIONS; PATHOLOGIC STUDY OF 47 CASES. William O. Russell,* R. Lee Clark, Jr., Michael L. Ibanez, and Edgar C. White, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

Forty-seven thyroid glands provided the material for a study of the intraglandular distribution of thyroid carcinoma and served to provide a basis for pathologic evaluation of the most effective form of surgical treatment for this condition. The entire glands, with contiguous lymph nodes when attached, were prepared and sectioned at 50 μ levels. No attempt was made to select the cases with respect to the stage of the neoplasm. The only requisites were that the diagnosis be established either by prior enucleation of an intraglandular mass or by biopsy of a cervical node, and that the whole gland or the gland minus the previously enucleated mass be available for study. In 57 per cent of the 47 glands the tumor involved either the contralateral lobe or isthmus, or one or more pericapsular nodes on the contralateral side. It was concluded from these findings that, in a significant number of cases, simple enucleation of a tumor mass or lobectomy would not eradicate all cancerous tissue. It was also apparent that the capsule of the gland provided the first barrier to the spread of tumor outside the gland, and that the pericapsular nodes on each side were the first sites of extraorgan extension.

These observations provide an anatomic explanation of the high incidence of recurrent thyroid carcinoma following lobectomy or enucleation of the tumor. The results indicate that the maximal opportunity for eradication of the lesion is afforded by total thyroidectomy with excision of the pericapsular lymph nodes. The pathologic anatomy of thyroid carcinoma will be discussed with respect to

(1) area variations in growth patterns in the different types of neoplasm; (2) the routes of intraglandular spread; and (3) the possible causative significance of associated conditions such as thyroiditis and thyroid adenoma.

FOLLICULAR AND PAPILLARY CARCINOMA OF THE THYROID: ANALYSIS OF 77 CASES. C. A. Hellwig,* V. E. Chesky, and J. W. Welch, Hertzler Clinic and Hertzler Research Foundation, Halstead, Kans.

There is a tendency to discard detailed histologic classifications of thyroid cancer and advise most radical surgical methods if a diagnosis of cancer has been made. The only type which generally is regarded as relatively benign is papillary carcinoma, while all nonpapillary tumors are given a poor prognosis. Crile pointed out that small areas of papillary growth in an otherwise follicular carcinoma allowed classification of such a tumor as a relatively benign lesion.

The 77 cases observed at the Hertzler Clinic presented an unusual opportunity to study the natural history of follicular and papillary carcinoma by clinical and pathologic methods, including a follow-up study over many years. Almost all cases came to observation without previous operation, and only limited surgical excision was performed. No postoperative therapy with deep x-ray or radioactive iodine was given in the majority of cases. The results indicated that pure papillomas and follicular variants of papilloma had the same favorable prognosis, confirming the viewpoint of Crile. However, it was also found that nonpapillary follicular carcinomas had almost as favorable a course after limited surgery.

For purposes of prognosis and indication of the extent of operation, the histologic classification of thyroid tumors remains of greatest practical importance. It can never be replaced by radioactive iodine tracer studies.

MALIGNANT LYMPHOMA OF THE STOMACH; ITS DIAGNOSIS, DISTINCTION AND BIOLOGIC BEHAVIOR. J. Leslie Smith, Jr., and Elson B. Helwig,* Armed Forces Institute of Pathology, Washington, D.C.

The purpose of this study is to help clarify the vague concepts regarding the biologic behavior and histologic nature of lymphoid lesions of the stomach. Interest in these lesions has been stimulated by the recognition that increasing numbers of patients with gastric lymphoma have survived for long periods of time, and by the difficulty encountered in the histologic distinction between malignant lymphoma of the stomach and reactive lymphoid hyperplasia.

One hundred thirty-one cases procured from the files of the Armed Forces Institue of Pathology were classified as gastric lymphoma. There were also 42 instances of benign gastric lesions characterized by varying degrees of reactive lymphoid hyperplasia. These served as the basis for this study. The extent of involvement, therapy, histologic features and duration of survival were correlated. Particular attention was devoted to 88 of the 131 patients with lesions classified as gastric lymphoma, who survived 5 years or longer. The histologic features of the benign gastric lesions with lymphoid hyperplasia were compared with those lesions which were considered to be malignant.

The following observations have been made: (1) Reactive lymphoid hyperplasia in the stomach may be mistaken for malignant lymphoma. (2) Certain histologic criteria may be used to distinguish between the two conditions. (3) The biologic behavior of malignant lymphoma, localized to the stomach, is distinctly different from that of lymphoma which involves the stomach as part of a generalized disease. (4) Evidence supports the concept that localized gastric lymphoma exists as an entity apart from generalized lymphoma. (5) It is possible that some of the localized lesions which appear malignant but behave in benign fashion are indeed hyperplastic lesions but have histologic features not easily distinguished from those observed in malignant lymphoma of the generalized type.

RELATION OF GASTRIC EPITHELIAL HYPERPLASIA TO CARCINOMA. Sheldon C. Sommers* and Geoffrey A. Boughton, Massachusetts Memorial Hospitals, Boston, Mass.

Among 168 necropsied cases of carcinoma of the stomach in which uninvolved gastric mucosa was available for study, an associated glandular epithelial hyperplasia was observed in approximately 80 per cent. Thirty-five cases (21 per cent) had an overgrowth of the superficial and gland-neck epithelium around the gastric pits, unaccompanied by other changes indicative of so-called "atrophic gastritis." In 40 cases (24 per cent) intestinal metaplasia was observed. This was associated with foci of degeneration and regenerative hyperplasia, alterations usually classified as "chronic atrophic gastritis." In 36 cases (21 per cent) both the superficial epithelial hyperplasia and the intestinal metaplastic and hyperplastic changes deeper in the gastric glands were present. Gastric polyps were observed grossly in 12 cases (7 per cent). In 3 cases (2 per cent) carcinoma arose adjacent to hyperplastic epithelium at the edge of a gastric ulcer. Gastric carcinoma *in situ*, merging with epithelial hyperplasia, was also found in 6 stomachs (4 per cent), the seats of invasive carcinoma elsewhere.

The presence of gross or microscopic epithelial hyperplasia in the gastric mucosa of the stomachs containing carcinoma in all except 19 per cent of the specimens investigated, is considered to indicate the likelihood that some forms of gastric epithelial hyperplasia have precancerous significance. Similar findings in noncancerous control specimens were less common. The use of the conventional term "chronic gastritis" applied to a wide variety of pathologic alterations of the gastric mucosa obscures the frequency with which gastric carcinomas may arise in association with focal or diffuse epithelial hyperplasia.

FIBROUS MESOTHELIOMAS, EXTRAOVARIAN THECOMAS AND PHYSIOLOGIC EFFECTS OF MESOTHELIOMAS INCLUDING HYPOGLYCEMIC AND NEPHROTIC SYNDROMES. Donald B. Nevius and Nathan B. Friedman,* Cedars of Lebanon Hospital and University of Southern California, Los Angeles, Calif.

Fibrous mesotheliomas and ovarian thecomas are histologically very similar. Instances of hypoglycemia associated with extraovarian thecoid mesothelial tumors are reported. Some of the spindle cell tumors reported in the literature as islet tumors because of hypoglycemia belong in this category. The relationship of this entity to Meigs' syndrome is discussed. The occurrence of a nephrotic syndrome in patients with epithelioid mesotheliomas is presented and discussed in terms of production of mucopolysaccharide and the relationship to osteroarthrotic and polyserous manifestations.

RADIATION INDUCED BRONCHOGENIC CARCINOMA IN RATS. Marvin Kuschner,* Sidney Laskin, Norton Nelson, and Bernard Altschuler, New York University-Bellevue Medical Center, New York, N.Y.

Squamous metaplasia of bronchial epithelium and squamous cell carcinoma of bronchial origin have been produced in the lungs of rats exposed to the beta radiation of the ruthenium-rhodium system (Ru^{106}-Rh^{106}). By means of an intrabronchial pellet implant technique, it was possible to expose animals to a range of doses from well defined sources. These graded doses were derived from plating the pellets with amounts of Ru^{106} which varied from 7×10^{-3} μc. to 14 μc. Cancers have appeared as early as 8 months at doses estimated at 2×10^{5} rads. Advanced squamous metaplasia was seen as early as 6 days (70 rads). The incidence data relating to the development of metaplasia and carcinoma is suggestive of a dose-response relationship.

The pellet technique has lent itself to a study of the serial stages in the morphogenesis of radiation-induced cancer of the lung. The alterations observed have included hyperplasia, metaplasia, and varying degrees of atypia proceeding to invasive carcinoma.

PRODUCTION OF LESIONS RESEMBLING CARCINOMA *in Situ* OF THE CERVIX UTERI. Otto Saphir,* and Michael L. Leventhal, Michael Reese Hospital, Chicago, Ill.

In patients scheduled for hysterectomy because of myofibromas, podophyllin was applied to the cervix of the uterus at various times. After hysterectomy, the treated region was examined microscopically and compared with the untreated portion of the cervix. There was severe disorganization of the entire epithelial cell layer with most bizarre cellular changes of the epithelium. These ranged from general atypism to marked anaplasia with an abundance of highly atypical mitotic figures. Similar changes had been produced on the skin of various experimental animals by the application of podophyllin and colchicine. However, in the cervix the lesions closely resembled those seen in carcinoma *in situ*, especially since the epithelial cells showed all the characteristic features of noninvasive malignant neoplasm.

TROPHOBLASTIC CELLS IN VAGINAL AND ENDOCERVICAL SMEARS: PROBLEMS CONCERNED WITH THEIR RECOGNITION AND INTERPRETATION. John T. Prior,* Eleanor Bechtold and Norbert B. Reicher, State University of New York, Upstate Medical Center, Syracuse, N.Y.

This study was initiated following an experience in which trophoblastic cells in an instance of abortion were nearly mistaken for malignant cells in a vaginal smear. Material for the study was gathered from routine screening smears obtained from 4,000 pregnant women. They were culled from a total of 55,700 cases of all types screened over a 9-year period. The great majority of the 4,000 smears were obtained in the first trimester of normal pregnancies. Complications included threatened and incomplete abortion, placenta previa, retained secundines, hydatidiform mole (including two cases of chorio-adenoma destruens) and two cases of chorio-epithelioma.

It was concluded that trophoblastic cells were exfoliated in all cases of mole and chorio-epithelioma, and were very rarely seen in instances of abortion and in normal pregnancies. Because of this, trophoblastic cells may easily be mistaken for atypical cells and may be a basis for false positive reports of neoplasm. This is an important matter, in view of the increased frequency with which microscopic cervical carcinoma is being encountered in women of child-bearing age.

This presentation includes a detailed analysis of the distinguishing characteristics of both normal and abnormal syncytiotrophoblastic and cytotrophoblastic elements. Other benign and malignant lesions with which they may be confused are discussed.

COMPARISON OF NUCLEAR MASS AND ALLIED PHENOMENA IN UTERINE CERVICAL SQUAMOUS MUCOSA: NORMAL EXOCERVIX, METAPLASIA, DYSPLASIA, INTRAEPITHELIAL CARCINOMA AND INVASIVE CARCINOMA. Alvan G. Foraker,* Baptist Memorial Hospital, Jacksonville, Fla.

Nuclear mass and allied phenomena have been compared in uterine cervical squamous mucosa in various patterns ranging from the normal exocervix to invasive carcinoma. Twenty cases each of squamous metaplasia, dysplasia, intraepithelial carcinoma and invasive squamous cell carcinoma were studied by interference microscopy. Blocks were selected to contain normal exocervical mucosa in addition to the characteristic abnormal epithelium as a control procedure. The optical path difference (in 2 mounting media of different refractive indices) of 10 normal and 10 abnormal squamous cells in each case was determined, the cells were photographed, and nuclear size determined planimetrically. The following were computed on normal and abnormal cell nuclei: refractive index, total particle thickness, mean area, dry mass per unit area, mean weight, percentage area occupied by nuclei in standard sample, and weight of nuclear material in standard sample.

Statistical comparisons between the findings in the various types of squamous cells is reported. Preliminary scanning of the data suggests that invasive carcinoma cells will not have a strikingly higher dry mass per unit area than normal cells. This work is the latest phase of a continuing study of chemical and physical properties of squamous cells of the cervix in various transition forms from normal growth to invasive carcinoma.

ANATOMY OF THE "COLLECTING VEINS" OF THE KIDNEY (WITH CONSIDERATION OF FUNCTIONAL ASPECTS). Peter P. Ladewig,* Laird Memorial Hospital, Montgomery, W.Va.

Histologic studies, plastic reconstructions, and vascular corrosion preparations showed that "collecting veins" of the human kidney were characterized by peculiar anatomic features: (1) Constricted segments in effluent branches close to their ostia; at these points concomitant arteries came into intimate contact with the venous wall. (2) Muscular fasciculi of an asymmetrically developed media which connected by streamer bundles (a) with the muscular media of the concomitant arteries mainly at the level of the constrictions; segments of complete side-to-side fusion of the two vessels over a single, common media were also observed; (b) with portions of the peripelvic muscles in the vicinity of the renal sinus. (3) Cushion-like agglomerations of modified muscular media which surrounded groups of cortical venules at their points of entrance into the collecting veins.

Constrictions of the lumen and valve-like muscular arrangements in the renal veins have been described in the literature. No record has been found of the other features cited. The observations strongly suggest the existence of a regulatory mechanism in the blood outflow bed of the human kidney as postulated by Homer W. Smith and supported by recent studies of other authors. Preliminary observations indicate that this regulatory mechanism is affected by various pathologic conditions.

BIOPSY STUDIES OF POST STREPTOCOCCAL ACUTE GLOMERULONEPHRITIS. Robert B. Jennings and David P. Earle, Jr., Northwestern University Medical School, Chicago, Ill.

This is a preliminary report of a study of the natural history of clinical acute glomerulonephritis in 18 patients who developed sporadic acute glomerulonephritis following hemolytic group A streptococcal infection. All but one of the patients were males, and all but two were under 40 years of age. Significant increases in antistreptolysin O, antistreptokinase or antihyaluronidase titers were demonstrated in each patient. In addition, group A hemolytic streptococci were recovered from throat cultures, and serum antibodies against type 12 hemolytic streptococci were demonstrated in a number of the patients. At the present time, 10 of the 18 patients have proteinuria 6 or more months after the onset of their diseases and are believed to have chronic glomerulonephritis. All patients had one or more percutaneous kidney biopsies. The histologic changes and the courses of the lesions will be described in patients with clinically healed and clinically persistent disease. Patients in whom the initial biopsy during the acute stage showed acute glomerulonephritis, at the present time show glomerular lesions whether clinically healed or not.

THE RELATIONSHIP OF PROTEINURIA TO TUBULAR ATROPHY AS STUDIED BY ENZYMATIC HISTOCHEMISTRY.† Max Wachstein* and Kurt Lange, Saint Catherine's Hospital, Brooklyn, and New York Medical College, New York, N.Y.

It is generally assumed that tubular atrophy in glomerulonephritis is caused by the diminished tubular blood supply which follows in the wake of the embarrassment of glomerular circulation. Data are presented which suggest that other

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factors may also be of significance. In rabbits in which nephritis was induced by potent duck anti-rabbit-kidney serum, the onset of proteinuria was characterized morphologically by the appearance of innumerable protein absorption droplets in the cytoplasm of proximal convoluted tubules and by the obstruction of distal tubular lumens by protein casts. Marked atrophy of proximal convoluted tubules occurred within a few days. Sustained proteinuria in rats appeared within 24 hours following a single intravenous injection of rabbit anti-rat-kidney serum, or repeated injections of stylomycin aminonucleoside (it was enhanced by DOCA). In contrast to the rabbit nephritis, the amount of protein droplet formation was only moderate, and casts were few. Tubular changes were less marked and focal in distribution. Depression of various enzymatic staining reactions was widespread in the rabbit kidney and only slight and focal in the 2 abnormal conditions of the rat. In general, hydrolytic enzymes, particularly alkaline phosphatase, were more depressed than oxidases (succinic dehydrogenase and DNA diaphorase), a pattern regularly seen in experimental hydronephrosis. Albuminuria appeared to be a contributory factor in the causation of tubular atrophy by the formation of casts and the creation of an internal hydronephrosis and in addition by the damaging effect of the protein absorption droplets on the tubular cytoplasm.

CORRELATION OF THE DEGREE OF GRANULATION OF JUXTAGLOMERULAR CELLS OF MAN WITH THE LEVEL OF PLASMA SODIUM AND WITH THE WIDTH OF THE ZONA GLOMERULOSA OF THE ADRENAL CORTEX.† James A. Pitcock and Phyllis M. Hartroft, Washington University School of Medicine, St. Louis, Mo.

Animal experimentation has shown that the degree of granulation of juxtaglomerular cells increases with dietary sodium depletion and can be correlated directly with the width of the zona glomerulosa of the adrenal cortex. In order to evaluate these factors in man, an unselected series of 200 kidneys from necropsies was surveyed using Bowie's staining technique to demonstrate juxtaglomerular cell granulation. The degree of granulation was estimated semiquantitatively by a technique described previously. Clinical records of all patients were examined to extract premortem determinations of electrolytes and blood pressure. Twenty-four cases were selected on the basis of availability of 3 determinations of plasma sodium within the last week of life. The average level of plasma sodium during this week and the width of the zona glomerulosa of the adrenal cortex were determined in these cases.

An inverse correlation was established between the degree of granulation of the juxtaglomerular apparatus and the average level of plasma sodium. A direct correlation was found between the degree of granulation and width of the zona glomerulosa of the adrenal cortex. No correlation was demonstrated between the degree of granulation of the juxtaglomerular apparatus and the blood pressure or levels of either plasma potassium or nonprotein nitrogen. Observations were made on the structure of the human juxtaglomerular cells and on changes which occurred in relation to age, race, and sex.

IMMEDIATE NEPHRITIS PRODUCED IN RABBITS BY DUCK ANTI-RABBIT-KIDNEY SERUM: LOCALIZATION OF THE ANTIBODY IN THE GLOMERULI.‡ Beatrice C. Seegal,* Margaret Bevans,* Konrad C. Hsu, and Mildred S. Rothenberg, College of Physicians and Surgeons, Columbia University, New York, N.Y.

Duck anti-rabbit-kidney serum injected into rabbits has been reported to produce nephritis after a latent period of several days. The present study describes experiments with duck anti-rabbit-kidney serum which produced an immediate

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nephritis. This parallels our previous experience in which relatively weak duck anti-rat-kidney serum caused a delayed nephritis in the rat whereas a strong serum induced an immediate nephritis. Ducks were immunized over a period of 21 months with suspensions of rabbit kidney. Interval bleedings were carried out, and the animals were exsanguinated after receiving a total of 17 gm. of rabbit kidney. Two to 3.2 ml. per kg. of body weight of pooled serums were injected intravenously into rabbits. Daily urine analyses were performed and interval blood urea nitrogen values were determined. At necropsy, 2 to 777 days following injection, tissues were taken for histologic examination. Some were rapidly frozen, sectioned in the cryostat and "stained" by the Coons' technique with rabbit anti-duck-globulin previously tagged with fluorescein isocyanate. Microscopic examination of these tissues under ultraviolet light revealed fluorescence in the areas where the injected antiserum had localized.

These experiments have provided the following results: (1) The pool of the terminal bleeding from the ducks immunized with rabbit kidney produced an immediate nephritis, as shown by proteinuria, nitrogen retention and renal lesions. (2) The disease was fatal in as little as 6 days or persisted for as long as 777 days. It healed in only one animal. (3) The injected duck antiserum localized in the glomeruli as demonstrated by the Coons' technique.

LOCALIZATION OF DUCK ANTI-RAT-LUNG SERUM IN RAT GLOMERULI ASSOCIATED WITH THE OCCURRENCE OF IMMEDIATE NEPHRITIS.† Ruth S. Triedman, Henry Metzger, Konrad C. Hsu, and Fred E. Urquhart, College of Physicians and Surgeons, Columbia University, New York, N.Y.

Antiserums to certain extrarenal tissue components, produced in heterologous species, will initiate glomerulonephritis when injected into members of the donor species. Rabbit anti-rat-lung serum has produced glomerulonephritis in rats and has been shown to localize in renal basement membranes when applied directly to sections of normal kidney. The following experiments were designed to study the nephritis produced in rats by duck anti-rat-lung serum. The course of disease, its histologic nature, and the *in vivo* distribution of injected antiserums were observed.

Nephritis was induced in 33 rats by injection of anti-rat-lung serum prepared in ducks. Daily records of proteinuria and weights were kept. Necropsies were performed 1 to 259 days following injection, and sections were fixed for examination. Rapidly frozen tissue was cut in the cryostat and "stained" with antibody to duck globulin coupled with fluorescein isocyanate according to the Coons technique. Microscopic examination under ultraviolet light revealed fluorescence in areas where anti-lung serum was retained.

The course of nephritis followed that seen after injection of rabbit anti-rat-kidney serum. Proteinuria was immediate in 23 of 33 animals, and nephritis occurred within 6 days in the remaining 10. The nephrotic syndrome was seen in 4. The 27 animals reviewed to date revealed renal lesions similar to those described as resulting from other nephrotoxins. The lungs did not show histologic changes attributable to the antiserums. Even after 259 days, tissues from 7 rats examined by the Coons method for antiserum localization revealed fluorescence in the basement membranes of all glomeruli, indicating the presence of antibody at this site. The lungs showed evidence of the effect of antiserum in one animal only.

AMINONUCLEOSIDE NEPHROSIS IN RATS. S. G. F. Wilson, Donald B. Hackel,* and Walter Heymann, Babies and Children's Hospital and Cuyahoga County Hospital, Western Reserve University, School of Medicine, Cleveland, Ohio.

The aminonucleoside of Puromycin regularly induced a nephrotic syndrome when injected into rats. Proteinuria appeared approximately 10 days after the

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beginning of a course of daily injections. Upon reinjection of the nucleoside into young rats, following recovery from the acute manifestations, a chronic renal disease could be induced which terminated in renal failure in 3 to 8 months. In the acute phase there were marked histologic changes in the renal tubules, and slight thickening of the glomerular basement membranes. The glomeruli in the chronic phase showed more prominent alterations, with marked fibrosis. Neither ACTH nor cortisone, given before or during a course of injections, prevented or ameliorated the disease. Suppression of antibody formation by x-irradiation did not prevent the onset of it. The introduction of serum from rats which had just contracted an aminonucleoside nephrosis, soon after the intravenous administration of nucleoside, did not shorten the latent period.

Dogs, rabbits, guinea pigs, and mice failed to develop renal abnormality after the injection of nucleoside. Rabbits, guinea pigs, and rats all excreted unchanged nucleoside in the urine and also other nucleosides derived from the original substance. Some of these degradation products have been identified, and one may be the compound responsible for the renal lesions.

THE EARLIEST LESION IN AMINONUCLEOSIDE NEPHROSIS: AN ELECTRON MICROSCOPIC STUDY.† James C. Harkin and Lillian Recant, Washington University School of Medicine, Saint Louis, Mo.

A nephrotic syndrome that functionally and morphologically bears a considerable degree of similarity to nephrosis in man has been induced in rats by daily injections of an aminonucleoside, 6-dimethylaminopurine-3-amino-d-ribose. Within a week these animals developed proteinuria, followed by hypoproteinemia, ascites and an extreme degree of hypercholesterolemia. In the acute syndrome some tubular but no significant glomerular alterations were observed by light microscopy. However, after several weeks of proteinuria there was thickening of glomerular basement membranes and proliferation of glomerular epithelium. Renal tubular epithelial lipid deposits were identified.

Electron microscopy revealed significant structural changes prior to the occurrence of proteinuria. The initial lesion was a degeneration of glomerular epithelial cells characterized by fusion of their foot processes, increase in intercytoplasmic vacuoles, and the appearance of smooth-surfaced profiles which might represent swollen degenerating mitochondria. No tubular changes were observed. At 8 days, shortly after the initial proteinuria, glomerular lesions were similar, but more advanced. Electron-dense cytoplasmic bodies were prominent within glomerular epithelial cells and also lay free within Bowman's space. In the chronic syndrome, distortion of the glomerulus occurred with endothelial changes and a distorted refolded basement membrane. This experimental model of the nephrotic syndrome offers an opportunity for study of sequential structural and functional changes.

GLOMERULAR LESIONS IN RABBITS WITH EXPERIMENTALLY INDUCED PROTEINURIA AS DISCLOSED BY ELECTRON MICROSCOPY. John T. Ellis, M.D.,* The New York Hospital-Cornell Medical Center, New York, N.Y.

In previous studies it was shown that rabbits given a single large intravenous injection of a saccharated iron oxide preparation (S.I.O.) regularly developed massive proteinuria after an interval of 5 to 7 days. As judged by light microscopy, the glomerular capillaries of the animals with proteinuria of 24 to 48 hours duration were devoid of marked structural alterations. In the present study of the glomeruli of rabbits with proteinuria of brief duration, electron microscopy disclosed striking alterations in the epithelial cells which have not previously been described under similar conditions.

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Three albino white rabbits weighing from 750 to 1,500 gm. were given 15 to 20 cc. of S.I.O. intravenously and 3 additional animals were given a similar quantity of the material divided into 3 injections over a 10 to 15 day period. Twenty-four hour urine specimens were collected and analyzed for protein and the rabbits were sacrificed 24 to 72 hours after the onset of proteinuria (900 to 1,500 mg. per day). The glomerular changes in all 6 animals were similar. There was partial or complete loss of the foot processes (podocytes) of the epithelial cells, sheets of epithelial cytoplasm remaining in their place. The adjacent basement membrane appeared normal, as did the endothelial cells except for the presence of varying numbers of iron-positive, cytoplasmic granules derived from the S.I.O. given intravenously. The significance of these changes in the epithelial cells, in terms of glomerular filtration, remains obscure. They bear a striking resemblance to the changes that have been reported by others in the epithelial cells of the renal glomeruli in children with the nephrotic syndrome.

PRODUCTION AND INHIBITION OF HYPERTENSIVE VASCULAR DISEASE IN THE RAT BY CORTICOSTERONE. Floyd R. Skelton, Louisiana State University School of Medicine, New Orleans, La.

If the secretions of the regenerating adrenal cortex play any role in the pathogenesis of adrenal-regeneration hypertension in the rat, it has been assumed that either corticosterone or aldosterone, or both, were involved since they are the principal corticoids in this species. Hence the investigation of the hypertensive properties of these substances has become important. In the present experiment 1 mg. and 5 mg. of corticosterone were administered daily to groups of uninephrectomized, salt-treated rats with intact adrenals, enucleated adrenals, and no adrenals for a period of 5 weeks. Severe hypertension occurred in all groups receiving the 5 mg. dose, but none was observed in any group receiving 1 mg. of the steroid. Adrenal regeneration was virtually abolished by 5 mg. corticosterone and partially inhibited by 1 mg. daily, the latter dose preventing completely the hypertensive disease which regularly develops during adrenal regeneration. These observations indicate that regenerating adrenals probably secrete less than 1 mg. of corticosterone daily, an amount incapable of producing hypertension in intact or adrenalectomized animals under conditions of this experiment. The results suggest that adrenal-regeneration hypertension can only be ascribed to corticosterone if the period of relative adrenal cortical insufficiency following enucleation somehow sensitizes the organism to the hypertensive potentialities which this steroid does possess.

RENAL FUNCTION DURING AND AFTER ACUTE HYDRONEPHROSIS IN THE DOG. Rafael Dominguez* and R. B. Adams, St. Luke's Hospital, Cleveland, Ohio.

By means of a constant intravenous infusion of radioactive diodrast, the blood activity was recorded continuously (a) for a control period, (b) during ureter obstruction, (c) during decompression and (d) after decompression. The obstruction was produced by saline infusion into the left renal pelvis with either constant flow or constant pressure. A method was developed to calculate the net renal diodrast extraction clearance during complete obstruction. The blood activity changes were analyzed in terms of renal extraction clearance, pyelovenous flow, and intrapelvic pressure. The bilateral reduction of diodrast extraction occurred within an hour of the beginning of obstruction. This reduction was more pronounced in the obstructed than in the unobstructed kidney, but in 3 of 20 experiments the opposite result was observed. No evidence of intrarenal diodrast back flow during obstruction was found. On decompression, at a pressure of 75 mm. Hg or below, the foramens opened more or less completely, and as concentrated urine poured into the pelvis, still under pressure, a rush of diodrast entered the

blood stream, producing a large surge in the blood radioactivity. The surge proved the existence of previously established pyelovenous channels, and the onset of the surge fixed the time of reopening of the foramens. After decompression, the evaluation of the excretory function of the previously obstructed kidney was complicated by varying degrees and combinations of (a) continued closure of the foramens, (b) persisting pyelovenous back flow, and (c) additional impairment of diodrast extraction. Methods have been developed to estimate the effects of these different factors.

URINARY CORTICOSTEROIDS IN HARTLEY STRAIN 2 AND STRAIN 13 GUINEA PIGS. E. M. Nadel,* B. G. Young and A. C. Hilgar, National Institutes of Health, Bethesda, Md.

Because of previously noted variations in the urinary excretion of corticosteroids when guinea pigs of different genetic backgrounds were utilized in various experimentally induced pathologic conditions, a study of the daily excretion of 3 unconjugated polar corticosteroids (6β hydroxycortisol, 2α hydroxycortisol and cortisol) was undertaken. This was carried out over a 24-day period in non-inbred (Hartley albino) as well as inbred (strain 2 and strain 13) guinea pigs, prior to and immediately following the administration of 10 units of ACTH, using established methods. Prior to ACTH treatment, the total daily excretion of these 3 free corticosteroids was 40γ, 63γ, and 48γ, while following ACTH the excretion was 285γ, 238γ, and 166γ for Hartley, strain 2 and strain 13 animals respectively. Prior to ACTH treatment, the major corticosteroid excreted by all 3 animal groups (10 to a group) was 2α hydroxycortisol (17γ, 33γ, 28γ). Following ACTH, the major polar corticosteroid excreted by non-inbred Hartley animals was cortisol (176γ) whereas strain 2 and strain 13 animals excreted less (64γ, 44γ). The major polar corticosteroid excreted by the inbred strains was 2α hydroxycortisol (strain 2, 103γ; strain 13, 86γ) while the Hartley guinea pigs excreted 75γ. Although the excretion of 2α hydroxycortisol as the major free normal polar corticosteroid was unanticipated, the high production of cortisol in this species has been well known and has been offered as partial explanation for successful homeotransplantation. It may also be linked to the ease of heterotransplantation to the eyes of this species, as well as to susceptibility to infection.

DIETARY PROTEIN AND HYPERTENSION OF DOG: PROTECTION BY URETERO-CAVAL ANASTOMOSIS WITH A STUDY OF KIDNEYS SO TREATED. E. E. Muirhead* and J. A. Stirman, the University of Texas Southwestern Medical School, Dallas, Texas.

Dietary protein potentiates renoprival hypertension of the dog. In the present observations a group of 10 dogs had one ureter connected to the vena cava and the opposite kidney removed. Although this group was treated precisely as the hypertensive nephrectomized group which received protein, the arterial pressure was not elevated. The kidneys from the group with uretero-caval anastomosis were studied. As compared to their normal partners, these kidneys increased in weight within 8 days by an average of +68 per cent. There was no hydronephrosis. The medulla appeared to enlarge more than the cortex. Mitotic figures were encountered more often in the medulla than in the cortex. Mitoses were least frequent near the capsule and most common in the outer medulla. Preliminary observations by dehydration and lipid extraction of the enlarged kidney indicated certain changes. Sampling the total renal structure indicated a slight increase in water content, a decrease in extractable lipids and an increase in nonlipid residue. When the cortex and medulla were analyzed separately, these changes, and particularly the increment in nonlipid residue, were more marked in the medulla.

Intact renal tissue unable to excrete externally appeared to provide protection against the development of hypertension. The increase in size, the prominent

mitotic activity and the marked increase in nonlipid residue in the medulla indicated the need to focus attention on this area as a possible site for the function(s) protecting against an elevation in arterial pressure.

SKELETAL LESIONS CAUSED BY BETA MERCAPTOETHYLAMINE IN RATS. P. Ramamurti and H. E. Taylor,* University of British Columbia, Vancouver, B. C., Canada.

Interesting lesions simulating idiopathic scoliosis, slipped epiphysis, dissecting aneurysm, and other mesenchymal abnormalities, have been produced by feeding laboratory animals beta amino propionitril ($NH_2CH_2CH_2CN$). This paper reports on the skeletal lesions produced by feeding young Wistar rats with beta mercaptoethylamine ($NH_2CH_2CH_2SH$). One group of rats received daily intraperitoneal injections of 20 mg. of mercaptoethylamine, and another group was given a diet containing 0.5 per cent mercaptoethylamine for 12 weeks. The former group of animals showed no abnormalities as judged by radiologic, gross pathologic, and histologic examinations, whereas the latter group developed severe skeletal lesions consisting of scoliosis, displacement through the epiphyseal plate, and bowing deformity of long bones. Histologic examination revealed widening and disorganization of the epiphyseal plate mainly due to an increase in width of the zone of proliferating cartilage with associated tears. Linear zones which were strongly PAS-positive occurred in the ground substance. The lesions superficially resembled those caused by beta amino propionitril, but with certain differences. Scoliosis and periosteal new bone formation were more marked with the latter substance, and the "zone of maturing cartilage" was involved primarily. The possibility that the lesions caused by mercaptoethylamine were attributable to the effects of its sulfhydryl group will be discussed.

SEX-LINKED HYPEROSTOSIS IN THE PARAKEET. Hans G. Schlumberger,* Medical Center, University of Arkansas, Little Rock, Ark.

Among approximately 1,000 parakeets *Melopsittacus undulatus*, necropsied during the past 3 years, 20 females aged 2 to 4 years had multiple hyperostoses; no male bore similar lesions. The skull, sternum, vertebrae, and sacrum were most often affected. The overgrowths ranged in size from 2 or 3 mm. to 3 cm. in diameter; some that arose from the retro-orbital bone led to exophthalmos. Nerve pressure from sacral exostosis produced leg paralysis. Histologically, some lesions consisted of dense bone; elsewhere they were composed of long osseous trabeculae, separated by connective tissue and bordered by solid rows of osteoblasts. Haversian canals were absent; cement lines were prominent.

Egg-laying hens normally produce large amounts of endosteal bone in the humeri and femora; similar changes were found in some of the birds with hyperostoses. This and the sex-linkage of the lesions suggest that an excess of estrogen may be a causative factor, but no consistent ovarian abnormalities were observed. Stilbestrol (4.5 to 7.0 mg.) induced endosteal bone formation. This was also accompanied by lipemia and hypercalcemia ranging to 75.5 mg. per hundred cc. of blood. In 16 birds, 4.5 mg. pellets of stilbestrol were introduced subcutaneously. Four of the 10 parakeets that survived over 100 days developed hyperostoses. Among 11 birds receiving 7 mg. of stilbestrol monthly and surviving over 100 days, 5 had hyperostoses; of 6 that received 3 mg. monthly and survived over 100 days, 3 developed hyperostoses. The lesions were usually limited to the sacrum, but in 2 cases nodules were present on each of 2 ribs. Though only 2 to 5 mm. in diameter, these hyperostoses resembled the spontaneous variety grossly and histologically.

SUBTOTAL (5/6) NEPHRECTOMY ON WEANLING RATS AND THE LONG TERM CHANGES IN THE BONES AND ENDOCRINE ORGANS.† Ashton B. Morrison, T. Robbins and Julius Gordon, Duke University Medical Center, Durham, N. C.

In 100 male weanling rats of the Osborne Mendel strain, a subtotal (5/6) nephrectomy was carried out, and sham operations were done on another group of 100 rats as controls. Both groups of rats were kept under identical conditions and fed the same diet. Since the experiment was started about 10 months ago, 27 of the experimental animals have died and a similar number of the controls have been killed. The tibias in the experimental animals revealed osteitis fibrosa and osteomalacia, but the most interesting findings occurred in the epiphyseal plates where alterations were similar to those seen in renal rickets in the human subject. This appears to be the first time such lesions have been produced in the experimental animal without a concomitant dietary modification. Adrenal hypertrophy was marked, but the pituitary was smaller than in the controls. The testes were greatly reduced in size, and the prostate and the seminal vesicles were also correspondingly much smaller than in the control groups. Medial calcification of the aorta and parathyroid hyperplasia similar to that described by Lehr were noted in many of the experimental animals.

GONADAL DYSGENESIS IN INFANTS AND CHILDREN. James B. Arey* and Angelo DiGeorge, St. Christopher's Hospital for Children, and Temple University School of Medicine, Philadelphia, Pa.

Although Turner's syndrome, gonadal dysgenesis occurring after puberty, is well recognized by endocrinologists, its frequency, manifestations, and pathogenesis are less familiar to pathologists. Moreover, relatively few reports have appeared concerning the occurrence of this condition in the prepubertal period; some of these have emphasized its association with the Bonnevie-Ullrich syndrome in infants.

The present report describes the clinical and pathologic findings in 6 infants and children who died and in whom the diagnosis of gonadal dysgenesis was established by histologic examination of the gonads. The phenotype of all of the patients was female. Five of the 6 were less than 6 months of age at the time of death, and in 4 of these death occurred as the result of severe congenital malformations. The diagnosis of gonadal dysgenesis or of Bonnevie-Ullrich's syndrome was considered during life in 3 of the 6 patients on the basis of the clinical manifestations or the difference in the chromosomal sex pattern and the phenotype of the infants. Variations in the histologic pattern of the affected gonads are described. The importance of determination of the chromosomal sex pattern during life or histologic examination of the gonads in all phenotypic females dying with severe congenital malformations is emphasized.

THE THYMOMA AND MYASTHENIA GRAVIS. Harvey Mendelow and Gabriel Genkins, The Mount Sinai Hospital, New York, N.Y.

A definite correlation between myasthenia gravis and thymic abnormalities, especially thymic tumors, is well known. It has been proposed that thymomas may act as the cause of myasthenia gravis through some hormonal mechanism as yet unknown. In an attempt to evaluate this concept, a series of 27 complete necropsies in patients with myasthenia gravis was examined. Thymic tumors were found in 10 of these cases. A parallel series of 18 surgically removed thymomas

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were also studied; 4 of these had been removed from patients with clinical evidence of myasthenia gravis. The thymic tumors in each series were classified on the basis of gross and microscopic features and with the aid of histochemical methods.

This study indicates that only a partial correlation exists between the histologic type of thymoma and the presence of myasthenia gravis. In a few of the cases, clinical evidence of myasthenia gravis was noted for the first time after partial or complete removal of a thymoma. Previously reported investigations are extended to demonstrate that the presence of a thymoma profoundly alters the severity of a case of myasthenia gravis and increases the frequency of myositis and myocarditis observed at necropsy.

EXPERIMENTAL THYROIDITIS IN RABBITS, GUINEA PIGS, AND DOGS, FOLLOWING IMMUNIZATION WITH SPECIES SPECIFIC THYROID EXTRACT. Kornel L. Terplan,* Ernest Witebsky,* and Noel R. Rose, University of Buffalo School of Medicine, Buffalo, N.Y.

Distinct inflammatory lesions were produced in the thyroid glands by using saline extracts of one lobe of the thyroid and injecting it, with equal parts (0.05 ml.) of Freund adjuvants, into the foot pads of animals. Similar results were obtained with injections of pooled thyroid extract and, in the rabbit, with purified thyroglobulin from the same species. The extent of the histologic changes paralleled somewhat the level of the antibody titer in the serum. About 40 per cent of the experiments in rabbits yielded positive results. In guinea pigs which received pooled guinea pig thyroid extract, the histologic changes of thyroiditis were nearly always present, although the production of antibodies was not as strong as in the rabbit. In dogs the thyroiditis was particularly severe, although no significant titer of antibody formation could be demonstrated. These experiments extended over the last 4 years and included about 140 rabbits into which a variety of organ extracts or thyroid extracts of different species were injected. There was also a large number of controls.

The histologic alteration of the thyroid gland resembled chronic thyroiditis, as observed in man, with focal or diffuse pattern. The following histologic features seemed to be of special significance: There was a distinct reduction in the number of thyroid follicles containing normally stained colloid. Pre-existing acini appeared completely masked by dense infiltrations of lymphoid cells. The surrounding follicles contained a variety of inflammatory elements and numerous disintegrating epithelial cells. In a majority of the cases having positive reactions to the extracts, impressive necrobiotic features were present within the lumens of the colloid-containing follicles surrounding the dense focal inflammatory infiltrations. In the thyroid glands of dogs, there was very marked hemosiderosis between the inflamed and atrophic follicles. Macrophages containing iron-positive granules were seen also in the follicular lumens of some of the rabbit glands. An eosinophil response was very pronounced in the stroma and follicles in the rabbit, scanty in the dog, and much less marked in the guinea pig. Extensive disintegration of follicle epithelium was clearly noticeable and combined with disruption of the follicle wall. Fairly large numbers of a variety of cells, including epithelium, macrophages, lymphocytes, and neutrophils were seen, especially in lumens where peculiar globular particles formed within the colloid. These stained only faintly with eosin. In the less severe stages, where the entire gland was not involved, the characteristic pattern consisted of focal areas containing small follicles with little or no colloid which were completely infiltrated and surrounded by lymphoid cells and eosinophils. Evidence of regeneration was characterized by nodular proliferation of thyroid epithelium, with no follicle formation. These formed tubercle-like adenomas. Mitotic figures could be seen in these nodules, and were also present occasionally within desquamated epithelial cells lying in the intrafollicular colloid.

THE PANCREATIC ISLETS OF RABBITS AFTER GLUCAGON ADMINISTRATION. B. W. Volk* and S. S. Lazarus, the Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, and the Albert Einstein College of Medicine, New York, N.Y.

The effects of long continued administration of glucagon, with or without cortisone, upon blood and urine glucose of rabbits, and upon pancreatic structure were studied. One mg. of crystalline glucagon (Lilly) was administered subcutaneously 3 times daily at 8-hour intervals, with or without the intramuscular administration of 1 mg. per kg. daily of cortisone acetate (Upjohn). Glucagon alone produced only a moderate degree of diabetes. On the other hand, glucagon with cortisone produced a marked hyperglycemic state. The degree of hyperglycemia usually became progressively more severe with increased duration of treatment up to 6 to 12 weeks, after which time it was ameliorated. The changes in the pancreas consisted of degranulation of B cells and glycogen infiltration into duct epithelium and B cells. In animals treated for prolonged periods, there was also neogenesis and hyperplasia of B cells. The B cell degranulation was thought to indicate an increase in insulin output from these cells as a result of hyperglycemia. The glycogen infiltration was one of the manifestations of the widespread increase in intracellular glycogen seen in diabetes. The B cell hyperplasia reflected a response of these cells to hyperglycemia with resulting increased insulinogenic capacity and improvement of the diabetes. An additional observation in most animals was a reduction in the number of identifiable A cells, and in many instances there were partly degranulated A cells. This apparent atrophy of the A cells suggested that glucagon was a hormone and that it was derived from these cells.

THE EFFECT OF FEEDING PANCREAS, PANCREATIN AND TRYPSIN IN RATS FED ETHIONINE.† Janis V. Klavins, Nathan Kaufman,* and Thomas D. Kinney,* Cuyahoga County Hospital and the School of Medicine, Western Reserve University, Cleveland, Ohio.

The effects of feeding pancreas, pancreatin, and trypsin to rats receiving diets containing ethionine were studied. The rats were fed a synthetic diet containing 18 per cent casein and 0.5 per cent dl-ethionine. Because of the ethionine in the diet, the animals showed pancreatic and liver damage as anticipated. The pancreatic lesions consisted of shrinkage and distortion of the acini, as well as some acinar regeneration. There was also a decrease in perinuclear basophilia and in the number of zymogen granules in the exocrine cells. Interstitial inflammation and slight interstitial fibrosis were noted. When the diet was supplemented by raw or boiled pancreas, the changes in the pancreas were essentially the same as in animals given the diet containing ethionine alone. When the diet was supplemented with trypsin or pancreatin, there was more evidence of regeneration. The individual acinar cells appeared larger, the cytoplasm contained vacuoles, there was less cytoplasmic basophilia, and the zymogen granules were fewer in number. There was also less interstitial fibrosis. Liver damage was manifested by small foci of necrosis, focal hemorrhages, regeneration of the liver cells, and slight bile duct proliferation. When the diet was supplemented by raw or boiled pancreas, the liver damage was less striking. On the other hand, when trypsin or pancreatin were added to the diet, the liver changes were more pronounced.

MYOPATHY IN ASSOCIATION WITH TOCOPHEROL DEFICIENCY IN CASES OF CONGENITAL BILIARY ATRESIA AND CYSTIC FIBROSIS OF THE PANCREAS. Tobias Weinberg,* Harry H. Gordon, Ella H. Oppenheimer and Harold M. Nitowsky, Sinai Hospital and Johns Hopkins Hospital, Baltimore, Md.

Extensive necropsy examination of muscle has been made in 3 patients with

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congenital biliary atresia and in 3 with cystic fibrosis of the pancreas. All of these patients had low plasma tocopherol concentrations, presumably as a result of faulty absorption of fat. In 2 of the 3 patients with biliary atresia and in 1 of the 3 with cystic fibrosis of the pancreas, there were lesions in muscle which closely resembled those of nutritional muscular dystrophy found in animals with tocopherol deficiency.

Specimens from 20 additional patients with congenital biliary atresia were also examined. Although no complete muscle survey could be made, satisfactory blocks of muscle were available from 6 of these patients. No lesions were found among these. In a similar retrospective survey of 48 patients with cystic fibrosis of the pancreas, satisfactory blocks of muscle were available in 10 cases. Dystrophic lesions were encountered in one of these patients. The failure to find the muscle lesions in some of the patients suggests the importance of conditioning dietary factors such as protein, pyridoxine, or vitamin A deficiency, as reported in the rat. The desirability of making extensive microscopic examination of the muscle in patients who die with malabsorption syndromes is indicated.

COMPARATIVE HISTOCHEMICAL PROPERTIES OF THE PERIODIC ACID-SCHIFF (PAS) REACTIVE MATERIAL OF ANTERIOR PITUITARY BASOPHILS, GAUCHER CELLS, THYROID COLLOID AND HEPATIC GLYCOGEN. Robert C. Bahn,* Griff T. Ross, and Robert W. Schmit, Mayo Clinic and Mayo Foundation, Rochester, Minn.

When paraffin sections of formalin-fixed anterior pituitary tissue of normal rats and man are subjected to the periodic acid-Schiff procedure, the basophils become intensely colored by a red pigment. To gain additional insight into the nature of the material responsible for this staining reaction, some histochemical properties of the PAS reactive material of anterior pituitary basophils were compared with those of a polysaccharide (hepatic glycogen), a mucoprotein (thyroid colloid), and a glycolipid (cerebroside of Gaucher cells). Lipid solvents such as anhydrous pyridine, acetone, and chloroform-methanol (1:1) were applied to tissues before and after formalin fixation. Paraffin sections of formalin-fixed tissues were subjected to the action of malt diastase and trypsin. The PAS reactive materials of the 3 model substances (hepatic glycogen, thyroid colloid, cerebroside of Gaucher cells) each responded differently to these procedures. However, the major component of the PAS reactive material of anterior pituitary basophils could not be differentiated from the glycolipid of Gaucher cells by these methods. These findings are not consistent with the current concept that the PAS reactive material of anterior pituitary basophils is a simple glycoprotein or mucoprotein hormone. Additional critical studies of the chemical and biologic nature of the cytoplasmic components of anterior pituitary basophils seem to be indicated.

IDENTIFICATION OF THE ANATOMIC DEFECT IN PSEUDOXANTHOMA ELASTICUM (PXE). Edwin R. Fisher,* Gerald P. Rodnan, and A. I. Lansing, University of Pittsburgh and Veterans Administration Hospital, Pittsburgh, Pa.

Pseudoxanthoma elasticum (PXE) is a hereditary disorder of connective tissue which commonly affects the skin, fundus oculi and cardiovascular system. The dermal lesion is characterized by a band-like arrangement of hematoxylin staining curlicues and granules within the mid-corium. The elastic tissue nature of PXE has been challenged recently; it has been proposed that the process primarily represents an alteration of collagen. Histochemical studies and fluorescence and electron microscopy were performed on sections of skin from 4 patients with PXE, 3 from the same kindred. The investigations revealed striking similarities between the fibrillary and granular components of the lesion and normal elastic fibers. Outstanding in this regard were their brilliant autofluorescence, lack of periodicity in ultramicroscopic preparations, weak or absent protein reactions, lability to elastase and pepsin. On the other hand, there were resistance to col-

lagenase and inhibition of the affinity for elastic tissue dyes following methylation. Their identification as altered elastic fibers was further enhanced by the accumulation of acid mucopolysaccharide, the presence of cross-bands in many of the fibers, and the deposition of calcium within the lesions. Identical alterations were demonstrated in apparently normal skin of a member of this kindred with overt manifestations of the disorder elsewhere. The absence of these features in the skin of members of the family in whom clinical evidence of disease was lacking indicated the recessive nature of the disorder. It appears that this abiatrophy of elastic fibers is, at least in part, dependent upon exogenous factors since the lesions classically occur at sites of flexural stress.

RELATIONSHIP OF AGE TO SWELLING PROPERTIES OF HUMAN DIAPHRAGM TENDON. Robert R. Kohn, Western Reserve University, Cleveland, Ohio.

The capacity of human diaphragm tendon to swell in aqueous solutions of HCl and NaOH was investigated. The tendon exhibited maximal swelling at pH 2.5, 12, and 14. Swelling at pH 2.5 and 12 was reversible and was probably of the Donnan equilibrium type, while at pH 14 the swelling was irreversible and was probably due to hydrolysis of bonds. The greatest increase in swelling was accomplished in 7 hours of exposure to the solutions. There was little change in swelling properties in tendon from subjects up to 30 years of age. A marked decrease in swelling capacity was apparent between the ages of 30 and 50, and a slow decline occurred after the age of 50. Calcium potentiated swelling at pH 2.5, but inhibited it at pH 12. Mechanical stress and irradiation *in vitro* had no effect upon swelling properties. Thermal denaturation alone caused tendon to swell as a function of age. Heat treatment also caused decreased ability to swell in acid, at rates which were related to age. Histologic studies showed a similar loss of ability to swell in the course of aging of pulmonary perivascular connective tissue. The nature of the swelling processes, their relation to physiologic age, and the possible role of thermal denaturation in aging are discussed.

HISTOLOGIC REACTION PATTERNS OF THE HUMAN SEBACEOUS GLAND. J. S. Strauss and H. Mescon,* Boston University School of Medicine, Boston, Mass.

Following different types of stress and injury to the sebaceous gland or the hair follicles of the human subject, certain patterns of response were observed in the sebaceous gland. The types of injury included plucking of hair, electrodesiccation of the follicle, and puncturing of the follicle. Various irritants, such as lipid materials extracted from the skin, colchicine and thallium acetate were injected.

The sebaceous gland was found to be stereotyped in its response to injuries in that the primary change was a replacement of the gland by cells which could not be differentiated from ordinary squamous cells. The sebaceous glands thus maintained their ancestral connection with the squamous cells of the epidermis, and even produced keratin. With inflammatory stimuli the sebaceous cells underwent proliferative changes which mimicked pseudoepitheliomatous hyperplasia. The relationship between these changes and those in spontaneous disease processes will be demonstrated.

RELATION OF BLOOD MAST CELLS TO TISSUE MAST CELLS; OF MASTOCYTOMA TO MAST CELL LEUKEMIA. S. Ono, J. Furth,* L. Zompetti, and P. Hagen, Children's Cancer Research Foundation, Children's Medical Center, Harvard Medical School, Boston, Mass.

A highly functioning mastocytoma has been carried in serial passages in the strain of origin (LAF_1 mice). The tumors grafted in the muscle reached a size of 5 to 10 gm. without metastasizing to distant organs. All tumor cells were granulated, even the dividing ones. The tumors contained large quantities of heparin,

histamine, and 5-hydroxytryptamine; these substances were apparently contained in the granules. The heparin values of the livers of tumor-bearing animals were within normal range. The histamine values were elevated. The intravenous injection of mast cells obtained from trypsinized tumors produced mast cell leukemia characterized by splenomegaly and hepatomegaly without enlargement of lymph nodes. Approximately 5 to 20 per cent of white cells in the blood were mast cells. Livers of mice with mast cell leukemia contained large quantities of heparin and enormous quantities of histamine. In tissue cultures, mast cells contained long filamentous and spider-like processes resembling those of microglia cells, but they also contained the characteristic granules.

Evidence suggests an essential identity of tissue mast cells and blood mast cells and a lack of close relationship with hematopoietic cells of the bone marrow and lymph nodes. Failure of animals with mastocytoma and mast cell leukemia to exhibit secondary generalized changes from the active substances contained in mast cells, suggests some homeostatic mechanism of release and maintenance of blood levels of these substances.

HISTOCHEMICAL CHANGES IN LIVER SUCCINIC DEHYDROGENASE DURING RAPID GROWTH FOLLOWING PARTIAL HEPATECTOMY. Bjarne Pearson,* Fred Grose and Rosa Green, Wayne State University College of Medicine, Detroit, Mich.

Male C_3H mice were subjected to hepatectomy and groups of animals were subsequently sacrificed at 21 hours, at 2 to 10 days, and at 14 and 28 days. Liver succinic dehydrogenase was investigated histochemically. Enzyme activities represented by formazan intensities were compared. The results were statistically significant. A decrease was present at 21 hours, and a further drop occurred from 2 to 10 days. Liver regrowth was complete by 10 days. Enzyme activity was not normal until after 14 days, but it was above normal at 28 days. In normal control livers, the enzyme was distributed as fine formazan granules only in the cells around portal areas for a distance of one third of the lobule, differing from DPN dehydrogenases, α-glycerophosphate, malic acid and lactic acid. The livers examined at 21 hours showed a marked change in the enzyme pattern. Large, ovoid and sausage-shaped formazan-positive globules, measuring up to 4 μ appeared in periportal cells. Their size diminished during the first 3 days. Complete restoration of enzyme pattern occurred only after 14 days. The enzymatic changes were demonstrated by a new tetrazole; 2,2′-diphenyl-5,5′-di-(m-nitrophenyl)-3,3′-(4,4′-biphenylene) ditetrazolium chloride. This formed fine noncrystalline formazan, and sections could be kept indefinitely. Lipids and phospholipids were removed by pretreating frozen sections with n-butanol/ether for 50 minutes at −65°C. This "split" the enzyme complex necessitating the addition of phenazine methosulfate.

NUCLEAR CHANGES IN THE LIVER OF THE RAT ASSOCIATED WITH DEVELOPING CIRRHOSIS.† J. W. Grisham and B. B. Banson, Washington University School of Medicine, St. Louis, Mo.

The existence of polyploidy in the parenchymal cells of the liver is well documented. Nuclei plotted according to their volumes fall into definite groups which are geometric multiples of the smallest group. In the normal liver usually only Class I, II, and III nuclei are found (diploid, tetraploid, and octoploid).

With the development of cirrhosis in the choline-deficient rat, nuclei shifted strikingly to higher ploidy classes as compared to pair-fed controls in which the shift was absent (Class I, 27.5 per cent; II, 62.5 per cent; III, 10.0 per cent). This phenomenon was first seen after about one month of deficiency when an increase in Class III nuclei was noted (Class I, 22.5 per cent; II, 54.5 per cent;

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III, 23.0 per cent). It reached a maximum after 3 to 4 months, just prior to the development of regenerative nodules, when Class III and Class IV nuclei were most numerous, and Class V and Class VI nuclei were present (Class I, 5.0 per cent; II, 10.5 per cent; III, 30.0 per cent; IV, 35.5 per cent; V, 14.0 per cent; VI, 5.0 per cent). In foci of nodular regeneration, lower ploid nuclei predominated with some increase in higher ploid nuclei as the nodules enlarged. Prior to the ploidy shift and after less than two weeks of deficiency, a persisting increase in nucleolar:nuclear ratio, nucleoli per nucleus, and total nuclear volume occurred.

EXPERIMENTAL FIBROPLASIA IN THE LIVER INDUCED BY CARRAGEENIN IN THE NORMAL RAT AND UNDER THE INFLUENCE OF CORTISONE AND ETHIONINE. F. G. Zak,* I. Bubelis and F. Paronetto, The Mount Sinai Hospital, New York, N.Y.

Carrageenin, a sulfated polygalactose extract from seaweed, produces a granulomatous tissue reaction with abundant collagen formation within a few days following intradermal and subcutaneous administration. The reaction disappears after several weeks. In the present investigation, 1 ml. of 0.1 per cent carrageenin was injected into the livers of normal rats just below the capsule. The animals were sacrificed at stated intervals over a period of 40 days, and the lesions studied microscopically, using silver impregnation, Weigert's stain for elastic fibers, and the chromotrope 2R aniline blue, Alcian blue, Astra-Blau, Rinehart-Abul Haj stains, Barlow's mast cell stain, periodic acid-Schiff reaction and toluidine blue O stain, the latter two with and without hyaluronidase digestion.

Acute necrosis accompanied by a neutrophil reaction appeared on the first day. On the second day macrophages, mobilized Kupffer cells and fibroblasts appeared in the exudate and subsequently became more abundant. As early as the second day some of the mononuclear cells became differentiated into mast cells which persisted in moderate numbers through the first 2 weeks. An amorphous ground substance, tinctorially suggestive of acid mucopolysaccharides, appeared after 4 days and increased during the first 2 weeks. Reticulum was deposited as early as the fourth day, as was PAS-positive extracellular material. Occluding venous granulomas were noted from the fifth day on. PAS-positive macrophages became increasingly numerous and were noted as pigment-containing elements. These cells were found also at a distance from the lesion. Duct cell proliferation was a part of the cellular response beginning on the second day. New elastic tissue was deposited from the fourth day on but only in small amounts. The time sequence, the speed of repair, and the intensity of this reaction were compared to the reactions in a similar series of animals which had received 5 mg. of cortisone twice weekly, and in a double series of rats receiving different dosages of ethionine in order to study the interplay between carrageenin fibrosis and ethionine duct cell hyperplasia.

STUDIES ON MECHANISM OF HEPATIC FIBROSIS. Hans Popper,* Fiorenzo Paronetto, and Hendrika van der Noen, The Mount Sinai Hospital, New York, N.Y.

The initial stages of hepatic fibrosis were studied in routine and one-μ thick sections prepared with silver impregnation, the periodic acid-Schiff reaction, and chromotrope aniline blue stain. Between liver cells and the lumens of the sinusoids, there is normally a fine, amorphous layer of PAS and aniline blue staining material, enforced by few reticulum fibers. Around ductules a continuous argentaphil membrane exists. Kupffer cells contain a few fine PAS-positive granules. About liver cells with cytoplasmic alterations, as in ethionine intoxication or human nutritional fatty liver with and without hepatocellular degeneration, the aniline blue staining layer appeared widened, its reaction to PAS was stronger, and it also stained with colloidal iron and Alcian blue. The reticulum fibers were increased in number, resembling continuous membranes as they surrounded the ductules

which were also considerably increased in number. In some instances, granules, positive to PAS and partly positive to colloidal iron, were found in the liver cells. The Kupffer cells, especially near duct cells, revealed many PAS-positive granules of varying size. Fibroblasts could not be recognized in such areas. In the portal tracts, increased numbers of ductules were surrounded by a dense PAS-positive reticulum layer, which was independent of the pre-existing thick collagen bundles. Here, too, there were cells containing PAS-positive granules.

Apparently some types of hepatic fibrosis develop around damaged liver cells and proliferated bile ducts without significant contribution of fibroblasts. An amorphous layer, in which reticulum seems to form, appears to be characterized by mucopolysaccharides. The possible fibroblast-like role of Kupffer cells and of the PAS-positive granules, some of which apparently were acid mucopolysaccharide, requires further study.

RELATIONSHIP OF THE INCREASED IRON STORAGE IN SECONDARY HEMOCHROMATOSIS TO MARROW ERYTHROPOIESIS. Geoffrey Kent and Hans Popper,* Hektoen Institute for Medical Research, Cook County Hospital, Chicago, Ill., and The Mount Sinai Hospital, New York, N.Y.

The structural changes of the liver, observed in several cases of secondary hemochromatosis appeared to be the result of iron overload. The high incidence of erythroid hyperplasia with maturation arrest in secondary hemochromatosis also suggested that the excessive iron storage might depend to a large extent upon the state of erythropoiesis. To test this hypothesis, the iron distribution in the liver and other organs was studied in a large series of disorders, including cirrhosis, congenital and acquired hemolytic anemia, pernicious anemia, myxedema, chronic infection, and malignant tumor. Whenever possible, observations on the bone marrow were also recorded and correlated. Primary hemolytic anemia was associated with an iron distribution in the reticuloendothelial cells, whereas in cases of pernicious anemia or anemia resulting from faulty iron utilization, a striking predilection of iron for the parenchymatous cells was noted. In general, an increased iron storage was observed in hyperplastic bone marrow whereas little stainable iron appeared in association with hypoplastic marrow. Only 16 of 200 cases of cirrhosis revealed an increased amount of stainable iron although anemia had been present in the large majority of cases.

The observations strengthen the hypothesis that bone marrow activity regulates or at least is synchronized with iron absorption, and that excessive absorption of iron with concomitant faulty utilization by the bone marrow leads to the accumulation of iron in parenchymatous cells. Excessive iron storage within the peripheral portion of the liver lobule, in turn, may predispose the tissue to injury and thus lead to secondary hemochromatosis.

THE EFFECT OF NUTRITIONAL FACTORS ON EXPERIMENTAL VIRUS HEPATITIS IN MICE. B. Ruebner and J. Bramhall, Dalhousie University, Halifax, N.S., Canada.

The virus used in the present experiments was M.H.V.$_3$ which uniformly causes a fatal hepatitis in weanling mice. Mortality decreases with age and is approximately 30 per cent at the age of 32 days in the strain of mice used. The virus preparation and the mice themselves were free from *Eperythrozoon coccoides* which greatly enhances the pathogenicity of the virus. In the first experiment the effect of choline deficiency on the hepatitis was investigated. The diet used was that of Himsworth (1944) which contained 50 per cent lard and 16 per cent casein. Two groups of mice were used. One group of 28 mice received the experimental diet. Another 29 mice were fed on a similar diet to which 0.5 per cent choline had been added. After 11 days on their respective diets both groups were inoculated intraperitoneally with the virus. The number of deaths in the 2 groups

was the same (21), and it was concluded that the fatty liver of choline deficiency was no more susceptible to virus hepatitis than the histologically normal liver of the animals receiving the choline supplement.

In a second experiment the effect of one of the experimental diets (containing 50 per cent lard, 16 per cent casein, and 0.5 per cent choline) was compared with that in animals fed with Purina Chow. Two groups of 28 mice were used. The mortality of the mice on the experimental diet was 21 in 28 compared with 11 in 28 in the control group. This difference was statistically significant. It was concluded that deficiency of a dietary factor, thus far unidentified, has an enhancing effect on virus hepatitis in mice.

MECHANISM OF HEMATOPOIESIS: HEMOPOIETINS IN NORMAL HUMAN PLASMA. Bernhard Steinberg,* Albert A. Dietz, Ruth A. Martin, and M. A. Atamer, The Toledo Hospital Institute of Medical Research, Toledo, Ohio.

Chemical fractionation, by methods to be described, of normal human plasma resulted in the separation of several components. With rabbits as test animals and with methods of administration outlined in other publications, factors were found which acted upon the bone marrow. Several specific hematopoietic reactions were obtained. One effect was an increase in the proliferation of megakaryocytes and a significant increase in the number of peripheral blood platelets (thrombopoietin). Another effect was on the development and maturation of granulocytes (G-leukopoietin). Another component of normal human plasma produced a generalized suppression of hematopoiesis. Electrophoretic patterns of the various plasma components were correlated with reactions in the hematopoietic mechanism. Preliminary clinical trials suggest an effect of one of the components upon the regulatory production of granulocytes in acute myelocytic leukemia.

EFFECT OF BONE MARROW INJECTIONS ON SPLENIC NUCLEIC ACIDS OF X-IRRADIATED RATS. R. M. Iammarino and M. Berenbom, Kansas University Medical Center, Kansas City, Kans.

In previous studies from this laboratory, nucleic acids were shown to decrease in liver, lung, thymus and spleen, following whole body x-irradiation. In these organs, only spleen DNA had a purine-pyrimidine composition different from that of nonirradiated controls. This study was undertaken to determine the influence of bone marrow treatment on the nucleic acids of rat spleen.

Holtzman rats received homologous bone marrow intravenously immediately after 400 r. whole body x-irradiation. At 2 and 7 days these rats (group A), together with suitable irradiated (group B) and nonirradiated control (group C) animals, were sacrificed. At both 2 and 7 days, the rats which received injections (group A) and irradiated control animals (group B) showed a decrease in splenic weight, and in the amount of RNA per spleen and amount of DNA per spleen, when compared with nonirradiated animals (group C). At 2 days no difference between groups A and B was noted. At 7 days, however, the spleen weight, and the amounts of DNA and RNA per spleen were higher in group A as compared with the irradiated control (group B) animals. Abnormal and identical molar base ratios of DNA were observed in both groups at 2 and 7 days.

These observations indicate that bone marrow treatment under these experimental conditions influences postirradiation recovery by a return toward preirradiation levels of splenic nucleic acid content, but had no effect on the presence of an abnormal spleen DNA composition.

CHANGES IN THE DEOXYRIBOSE NUCLEIC ACID (DNA) SYNTHESIS OF BONE MARROW FROM RABBITS AFTER X-IRRADIATION. (A MICROSPECTROPHOTOMETRIC STUDY). Edwin M. Uyeki, Cecilie Leuchtenberger and Paul R. Salerno, Western Reserve University, Cleveland, Ohio.

The influence of ionizing radiations as studied biochemically by other workers

on whole tissues and cell populations of bone marrow have indicated variable effects on DNA synthesis. Since these biochemical studies yielded only computed average DNA values, one cannot ascertain whether or not the DNA synthesis of individual cells was changed after x-irradiation.

This report deals with an attempt to assess directly the DNA content of individual cells after x-irradiation. For this study, a special technique—Feulgen microspectrophotometry—was utilized; the method permits the DNA analysis in single cells in microscopic sections of bone marrow *in situ*. The DNA content of interphase nuclei of the femoral marrow cells from hemi-body x-irradiated rabbits was compared with the shielded femoral marrow cells from the same rabbit and with nonirradiated femoral marrows from normal rabbits. In contrast to the DNA synthesis regularly observed in at least 85 per cent of the normal interphase nuclei of immature bone marrow cells, the comparable cells of the x-irradiated bone marrow showed a marked decrease in DNA synthesis. A slight reduction was indicated as early as 6 and 12 hours after irradiation and a marked reduction was found at 24 hours.

STUDY OF DYNAMICS OF GASTROINTESTINAL EPITHELIAL PROLIFERATION BY DNA RADIOAUTOGRAPHS.† George Brecher,* E. P. Cronkite, H. Quastler, and V. P. Bond, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md., and Brookhaven National Laboratory, Upton, N.Y.

Thymidine labeled with tritium is rapidly incorporated into DNA, and provides a nuclear label of cells in the phase of DNA synthesis. The individual cells could be identified and turnover studied in radioautographs of animals sacrificed at intervals after a single injection of tritiated thymidine. DNA turnover did not occur, and the intensity of the nuclear label remained constant until a subsequent division during which chromosome duplication resulted from new formation of unlabeled DNA. Following division, labeled and unlabeled chromosomes were distributed at random between the daughter cells, and on the average each would contain one half of the original label.

In one group of mice, labeled cells were present within 15 minutes after injection into the generative regions of the gastrointestinal tract, i.e. the basal cells of the forestomach, the cells in the neck of fundic glands, and the cells at the base of the crypts of the pylorus, small intestine and colon. Labeled mitoses were numerous in all gastrointestinal epithelium studied within 1 hour. The intensity of the label remained undiminished in the majority of labeled cells in the colon as they moved toward the mouths of the crypts during a 5 day period of observation. However marked dilution of label occurred in the small intestine. Here the majority of labeled epithelial cells had been shed into the lumen by the 4th day. In the fundus, most labeled cells moved toward the mucosal surface, and only a few moved toward the base of the gland. Successive radioautographs permitted estimates of duration of DNA synthesis, of mitotic time, and of the time interval between them. This interval between DNA synthesis and mitosis varied from 30 minutes to several days in individual cells.

A STUDY OF THE ALTERATIONS IN PROTEOLYTIC AND PROFIBINOLYTIC ACTIVITY OF THE PLASMA IN VARIOUS DISEASE ENTITIES. Marvin Murray, University of Wisconsin, Madison, Wisc.

Experimental evidence has shown that fibrinolytic activity might be evaluated by measuring the esterase activity upon a suitable synthetic substrate. The substrate used in these experiments was Tosyl arginine sulfonyl methyl ester (TAME). This substrate is specific for fibrinolysin, trypsin, and thrombin, although at dif-

† Supported in part by the United States Atomic Energy Commission.

ferent reaction rates. Aqueous thrombin (0.3 ml.) was added to 3.0 ml. of citrated plasma. The fibrin clot was wound out and the plasma allowed to stand for 10 minutes in order to inactivate the thrombin. Aliquots (1.0 ml.) were added to two tubes: (a) containing 2.5 ml. tris buffer, 0.5 ml. H_2O, and 1.0 ml. TAME; and (b) containing 2.5 ml. tris buffer, 0.5 ml. (5,000 units) purified streptokinase, and 1.0 ml. TAME. These were allowed to incubate at 37°C. Aliquots (1.0 ml.) were taken at 0, 15, 30, and 45 minutes, and were added to 1.0 ml. basic formaldehyde. Titration of the acid liberated was carried out with 0.05 N NaOH. Evaluations in 31 normal individuals revealed a rather narrow range of activity, the means being: proteolytic activity, 2.4 μM acid per ml. and profibrinolysis 5.1 μM acid per ml. A similar evaluation of plasma from 46 patients with various diseases indicated that a pattern for increased proteolytic activity might indeed exist. From the results of this survey it would appear that major changes in proteolytic activity of the plasma occur in severe pulmonary disorders (neoplasm, granuloma), complications of the last trimester of pregnancy, and pancreatic disease (neoplasm, pancreatitis, mucoviscidosis). A detailed discussion of these changes is given.

THE CORRELATION IN THE RAT OF AGE WITH ITS DEGREE OF RESISTANCE TO ANTHRAX. Martha J. Taylor, George H. Kennedy, and George P. Blundell,* U.S. Army Chemical Corps, Fort Detrick, Frederick, Md.

The adult rat usually survives large doses of highly virulent *Bacillus anthracis* spores. It was discovered during an evaluation of this resistance that young rats succumbed to comparatively small doses of spores. The wide contrast in lethal dose response makes the maturing rat a fortunate species in which to study the influence of age on the development of natural resistance. Fischer 344 rats served as a source of genetically uniform hosts. The ages selected were accurately recorded to within 12 hours and included 4 groups between birth and 6 weeks. *B. anthracis* spores, selected for high virulence, were injected subcutaneously. Data from the groups of rats receiving 10^4, 10^7, 10^8, and 10^9 spores permitted the preparation of a group of curves recording death. The response to these spore doses depended upon the age of the rat at the time of inoculation. Ninety to 100 per cent of rats 0 to 5 days of age were killed by 10^4 spores, while half of those 6 to 8 days old survived this dose. The dose of 10^7 spores resulted in the death of half of the 25-day-old rats. Similar results were obtained when 10^8 spores were administered on the 29th to 31st day. An almost complete reversal of the response observed in the newborn rats occurred in rats 34 to 41 days old. Eighty-three to 100 per cent of this oldest age group survived inoculation with 10^9 spores. This study included 948 rats from 106 litters.

Symposium on Natural and Acquired Factors in Resistance to Disease

Referee (by invitation of the Council): W. Barry Wood, Jr.

Defects in Leukocytic Defenses in Skin Windows in Disease. J. W. Rebuck,* R. W. Monto, and E. A. Monaghan, Henry Ford Hospital, Detroit, Mich.

In human control subjects the leukocytic responses to nonpyogenic antigens consisted of 4 successive but overlapping phases dominated by neutrophils, lymphocytes, hypertrophied lymphocytes, and macrophages, in that order for the first 24 hours. In subjects with a positive reaction to the tuberculin test, the hypersensitivity-carrying lymphocytes were inapparent for 24 hours or more. In contrast, in Boeck's sarcoid there was a precocious macrophage response in 6 to 12 hours. In periarteritis nodosa there was a precocious lymphocytic and eosinophil infiltrate. In disseminated lupus erythematosus, LE cells appeared at times without the addition of plasma to the lesion and at times only after the addition of further necrotizing factors from the affected patient's plasma. In hypogammaglobulinemia there was depression of the two lymphocytic phases. This was also noted in a series of patients with Hodgkin's granuloma. In addition there was almost complete depression of leukocytic verdoperoxidase activity in lesions in Hodgkin's disease. In chronic lymphocytic leukemia the leukocytic phases were quantitatively intact, but there was occasional suppression of sudanophilic transformation in the lymphocytes. In subacute monocytic leukemia there was substitution of monocytes for lymphocytes in the inflammatory cycle. The human skin window affords an index of individual variability in leukocytic defenses in disease.

The Healing Wound as a Chemical System: A Histochemical Study.† J. F. A. McManus* and C. H. Lupton, University of Alabama Medical Center, Birmingham, Ala.

Slide preparations of human cutaneous wounds were prepared by the method of Rebuck and Crowley (1955) at intervals up to 60 hours after wounding. Biopsy specimens of similar wounds were made at intervals of 3 and 5 days after injury. Older human wounds were available from surgical procedures and necropsies. This tissue was compared with earlier material procured from wounds in guinea pigs and rabbits, under a variety of experimental conditions. All specimens were subjected to a number of histochemical studies, especially those concerned with complex carbohydrates. A number of the methods had not been applied previously in this situation. Initially, the cellular inflammatory reaction was characterized by fibrin and a predominance of neutral polysaccharide. This was followed by an acid mucopolysaccharide (AMP) phase which terminated with the appearance of capillary growth and fibroplasia. The latter was conspicuous by the fifth day. The sealing of the wound by crust formation set up anaerobic conditions in which the breakdown of damaged tissue and of the components of the exudate furnished the elements from which the tissue was reconstituted. In these studies the process of wound healing appeared to summarize in remarkably efficient fashion the various phenomena associated with growth in individual cells wherever studied. The energy component was supplied by carbohydrate (in the wound, the PAS-positive materials) and phosphate-bound elements were liberated by enzymes of the phosphatase group (alkaline phosphatase and 5-nucleotidase

† Earlier studies supported by Surgeon General, U.S. Army. Current studies under grants of National Institutes of Health.

might be considered representative of these). Cellular activity was evidenced in individual cells by large nuclei and by cytoplasmic basophilia, features indicative of protein synthesis. This was also demonstrated by new collagen fiber production. The contribution of the adjacent tissues to the wound area was characterized by the migration of cells and local edema, and the influence of the general metabolic state in the repair of the wounds was demonstrated by the effects of infection, cortisone administration, adrenalectomy and hypophysectomy.

THE COURSE OF NATURAL BACTERIAL INFECTION IN EXPERIMENTAL FROSTBITE OF RABBITS.† J. P. Kulka,* W. G. M. Hardison, P. Fremont-Smith, and G. J. Dammin,* Harvard Medical School and Peter Bent Brigham Hospital, Boston, Mass.

Antibacterial prophylaxis is widely practiced in the management of frostbite. Yet the contributory role of infection to final tissue loss has received little systematic study. In most lesions, both clinical and experimental, the usual manifestations of bacterial invasion are slight or absent. We investigated this problem because of the unexplained variability in the extent of gangrene in rabbits which had been subjected to freezing of a depilated hind foot by exposure to air at −25°C. The injured foot was protected from self-amputation. Bacterial cultures were made daily of subcutaneous tissue fluid and were found to become positive in all instances within 1 to 3 days after the exposure. Invasion apparently occurred from multiple cutaneous sites since the onset of infection in individual toes and the metatarsus often differed by 1 to 2 days, and organisms isolated from these regions were frequently not identical. The organisms were usually of low virulence and, despite increasingly heavy growth, suppuration was uncommon. Gangrene was usually of the dry type, and histologic evidence of bacterial disease was not prominent. No relationship between the onset or extent of gangrene and the type or severity of infection was noted. Nevertheless, in rabbits made severely leukopenic by nitrogen mustard, gangrene began earlier and was more extensive.

The observations indicate that the presence of "silent" infection in frostbitten tissue constitutes a variable in the course of the lesion which cannot be disregarded. The profound circulatory impairment associated with cold injury is probably a factor not only in lowering local resistance to bacterial invasion but also in suppressing the inflammatory response.

ACTIVATION OF QUIESCENT MUCORMYCOTIC GRANULOMAS IN RABBITS BY INDUCTION OF ACUTE ALLOXAN DIABETES. Walter H. Sheldon* and Heinz Bauer,* Emory University School of Medicine, Emory University, Ga.

The effects of a severe acute metabolic alteration on host resistance to infection were studied by producing acute alloxan diabetes in rabbits with subcutaneous granulomas induced by the Phycomycete, *Rhizopus oryzae*. Fungous granulomas thus formed in 17 metabolically normal rabbits remained confined to the inoculation site, showed no fungus proliferation, became sterile after 70 days and healed spontaneously. Acute alloxan diabetes with acidosis induced in 37 rabbits 8, 10, and 15 days after fungus inoculation caused a change in the morphologic appearance of the pre-existing granulomas. Proliferation of the fungus was noted in the center of the lesions in all these animals. In addition, focal necrosis of the granuloma wall was found in 22 rabbits. Extension of the fungus to adjacent tissues and blood vessel invasion occurred in 6 animals. There was no involvement of regional lymph nodes or other organs. These alterations in the lesions were not present in 5 rabbits with chronic alloxan diabetes without acidosis;

† Supported by Contract DA-49-007-MD-645 (Research and Development Division, OSG, DA) and Grant H-2206 from the National Heart Institute, National Institutes of Health, United States Public Health Service.

these were not included in this series. The observations indicated that the character of pre-existing mucormycotic granulomas could be drastically affected by a severe alteration of host metabolism such as occurred in acute alloxan diabetes. Moreover, the breakdown of granulomatous lesions and the concomitant activation of the infection which appear in a variety of infectious granulomas in man, may be reproduced and studied in chronic experimental mucormycosis.

PATHOGENESIS OF METASTASIS FORMATION OBSERVED *in Vivo* IN THE RABBIT EAR CHAMBER. Sumner Wood, Jr., School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.

A special adaptation of the rabbit ear chamber technique was employed to observe under high magnification and to photograph *in vivo* the intravascular behavior of cancer cells. Trypan blue-stained V2 carcinoma cells were injected into the auricular arteries of rabbits having vascularized ear chambers. Serial cinephotomicrographic records served as a permanent documentation and were particularly valuable in the objective analysis of the cellular phenomena.

Systematic observations of the fate of blood-borne cancer cells *in vivo* led to the following conclusions: Cancer cells passed rapidly from the arterioles into the capillary bed, where they became firmly adherent to the endothelium. The initial endothelial adherence appeared to be independent of capillary diameter, leukocytic sticking, vasomotor activity, or rate of blood flow. After a few minutes, minute thrombi slowly enmeshed the cancer cells. Cancer cells destined to penetrate the endothelium remained firmly adherent. Within 30 minutes to several hours, the endothelium subjacent to the cancer cells was altered. Leukocytes migrated down the static vessel and wandered at random over the endothelial surface before emigrating through it. Penetrating leukocytes appeared to leave endothelial defects behind, and through these, other leukocytes and, later, cancer cells emigrated. The earliest endothelial penetration by tumor cells was observed within 3 hours. Tumor growth in the extravascular tissue was progressive. Capillary buds arising from pre-existing vessels and entering the tumor were noted at 24 hours; by 57 hours the tumor contained a rich capillary bed. Histologic examination of fixed sections showed the characteristic V2 carcinoma.

The value of this new approach in the study of the intimate mechanisms of lodgment, endothelial penetration, and growth of embolic cancer cells, and the prevention of hematogenous metastasis is currently under investigation.

THE CYTOTOXIC ACTION OF NORMAL HUMAN SERUM ON CERTAIN HUMAN CELLS PROPAGATED *in Vitro*. Robert P. Bolande, Western Reserve University, Cleveland. Ohio.

Normal, human serum was found to be markedly toxic to 2 strains of atypical, human cells propagated serially in a medium containing normal horse serum (U12 uterine fibroblast and HeLa cell). It had no effect on relatively normal, short-term cultures of newborn foreskin fibroblasts. The toxicity of various serums was manifested by 100 per cent cytolysis of cells within one hour at 37°C in dilutions up to 1:64. These effects could not be produced with rabbit, guinea pig, horse, or rat serums. Since the cytotoxicity could be eliminated by heating the serum to temperatures greater than 45°C, the reaction was studied from the viewpoint of the complement-properdin system of serum. The reaction did not require the presence of properdin or intact C′ (complement), since RP (serum made deficient in properdin but containing C′), and RI (serum made deficient in C′1, the first component of C′) were as toxic as whole serum. R2 (serum deficient in the second component of complement, C′2) was somewhat less toxic, while R3 and R4 (serums deficient in C′3 and C′4) were essentially nontoxic. Recombinations of R3 and R4 re-established the toxicity, suggesting that the reaction required the presence of C′3 and C′4. Hemolytic titrations for the 4 components of complement (C′1, C′2,

C′3, and C′4) after heating and complement fixation of serum, also suggested that the toxicity was related to the presence of C′3, C′4, and possibly C′2. The reaction did not appear to require Ca^{++} or Mg^{++} ions. Thus, it was possible to demonstrate the sensitivity of certain atypical human cells to factors in human serum, which appeared distinct from properdin but which resembled in their properties 2 and possibly 3 of the 4 recognized components of complement. It is possible that these factors may differ from the usual components of complement, and that additional factors, as yet undefined, may participate in the cytotoxic system.

TRAPPING OF "SCHISTOSOMULA" IN THE LUNG OF *Macaca mulatta* WITH EXPERIMENTALLY INDUCED RESISTANCE TO *Schistosoma mansoni* INFECTION; PATHOLOGIC STUDY. Francisco V. Lichtenberg and Lawrence S. Ritchie, School of Tropical Medicine, University of Puerto Rico, and Army Tropical Research Medical Laboratory, Ft. Brooke, San Juan, P.R.

In an attempt to identify the mechanisms of resistance, 9 adult monkeys were immunized against *S. mansoni* by diverse infection schedules within an average period of 500 days. Resistance was tested by a massive standardized reinfection. Finally, animals were exposed to 5 times the lethal dose of cercariae. The path and fate of "schistosomula" were studied by serial skin biopsy, lobectomy biopsy and complete necropsy at appropriate intervals. Included in the study were step histologic sections for semiquantitative study and flushing of the portal circulation for a count of adult forms and larvae. Additional procedures included serial stool egg counts, complement fixation, cercarial agglutination, circumoval precipitin, and intradermal tests. Nonimmunized control monkeys inoculated in identical fashion died of massive infection within 56 days.

Small numbers of cercariae were retained in the skin of test and control monkeys. All of the immune monkeys and none of the controls had typical "penicilliary" foci surrounding between a third and a half of the intrapulmonary "schistosomula" on the sixth to eighth day. The larvae appeared imprisoned in precapillary pulmonary vessels with marked endothelial swelling and were surrounded by recent, well circumscribed, pneumonic foci. Some larvae showed degenerative changes. A detailed description of this lesion is given for the first time, and the reasons for considering it as a trapping mechanism are discussed. The reduced number of larvae reaching the liver hilus were retarded in development; most of them did not complete development or produce eggs, and they disappeared within 3 months, leaving minimal residua only. A general scheme of the morphologic immunity phenomena is outlined and alternative explanatory hypotheses are discussed briefly.

CROSS RESISTANCE TO CEREBRAL TYPHOID INFECTION AND INFLUENZA VIRUS NEUROTOXICITY. Robert R. Wagner and Edward W. Hook, the Johns Hopkins University School of Medicine, Baltimore, Md.

Mice receiving intracerebral inoculations of *Salmonella typhi* or large doses of non-neurotropic influenza virus often developed characteristic tonic convulsions and died in 48 to 72 hours. Salmonellas multiplied rapidly to high titer in brain tissue, whereas proliferation of influenza virus at this site was extremely limited. Despite marked differences in pathogenicity and biologic properties of these microorganisms, resistance to both infectious agents could be induced in strikingly similar fashion. Animals receiving intracerebral injections of typhoid vaccine 24 hours previously, resisted challenge infection with either *S. typhi* or influenza virus to about the same degree. Injection of subtoxic doses of influenza virus also protected mice against virus neurotoxicity although pretreatment with virus had only a minimal effect on cerebral typhoid infection. The most pronounced refractory state was produced by purified bacterial lipopolysaccharides prepared from *Escherichia coli* or *Salmonella abortus equi*. These substances inhibited bacterial multi-

plication in the brain markedly and protected mice against 200 LD_{50} of *S. typhi*. The route of inoculation was also important. Mice given intraperitoneal injections of 10μg. of *E. coli* lipopolysaccharide were fully susceptible to intracerebral challenge, whereas 0.01μg. of polysaccharide given intracerebrally afforded effective protection against both cerebral typhoid infection and influenza virus neurotoxicity. Resistance to either typhoid infection or virus toxicity developed in a few hours, was maximal at 24 hours, and gradually disappeared in about a week. Tolerance in rabbits to the pyrogenic effects of influenza viruses developed in similar fashion, additional evidence for a general phenomenon of acquired resistance unrelated to specific immunity.

NATURAL AND ACQUIRED RESISTANCE TO BACTERIAL INFECTIONS: SOME COMMENTS ON PRINCIPLES. W. Barry Wood, Jr. (Referee†), Johns Hopkins University School of Medicine, Baltimore, Md.

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THE EFFECT OF ANEMIA, HYPERTENSION, AND ANEMIA WITH HYPERTENSION UPON HEART WEIGHT. Tom D. Norman* and Robert D. McBroom, University of Mississippi Medical Center, Jackson, Miss.

Preliminary studies from this laboratory demonstrated a significant increase of heart weight in moderately anemic rats. To learn more of the mechanism of cardiac hypertrophy, a study was made of the effect of anemia and hypertension, singly and in combination, upon the rat heart. Forty-six Holtzman rats were used. In 22, unilateral nephrectomies with capsular stripping and the introduction of a silk figure-of-eight ligature or a bag around the remaining kidney were performed. Anemia was maintained for 8 weeks by the administration of phenylhydrazine in 12 of the animals operated upon and in 16 of those not operated upon. It was assumed that any increase of heart weight in the nonanemic animals which had been operated upon, and, in part, any such increase in the anemic operated rats was due to hypertension.

In groups with comparable terminal body weights, *t* tests were used to compare differences between the heart weight to body weight ratios. Differences between controls and anemic operated rats were significant ($P = 0.01$), and also between controls and anemic unoperated or nonanemic operated animals ($P = 0.05$). There were no significant differences between nonanemic operated and anemic operated rats ($P = 0.1$) or anemic unoperated and anemic operated animals ($P = 0.2$). These experiments indicate that the hypertensive effects on anemic cardiac hypertrophy were less than additive. There were no significant differences (analysis of variance) in the myocardial water content or blood urea nitrogen concentration of rats in the various groups.

CORONARY ATHEROSCLEROSIS IN NEW ORLEANS VERSUS GUATEMALA. Jack P. Strong, Carlos Tejada, Henry C. McGill, Jr.,* and Russell L. Holman,* Louisiana State University School of Medicine, New Orleans, La., and the Institute of Nutrition of Central America and Panama (INCAP), Guatemala.

Coronary arteries and aortas were collected from 150 routine necropsies in hospital and medicolegal services in New Orleans and Guatemala and prepared for study as previously described. In each geographic area there were 15 cases representing each of the 5 decades between 30 and 79 years of age. Coronary atherosclerosis in New Orleans was compared with that in Guatemala by ranking the coronary cases in each decade on the basis of overall severity of involvement. The New Orleans specimens were more severely affected in all age groups,

† By invitation of The Council.

the difference being insignificant at 30 to 39 years; moderately significant at 40 to 49 years; and highly significant after 50 years of age. Correlation between aortic and coronary lesions was determined by independently grading the aortas and coronary arteries as to severity of lesions in each decade. There was positive correlation between atherosclerotic lesions in the two vessels in each decade in the New Orleans material; however, there was positive correlation in the Guatemalan cases only in the 50 to 59 year group.

Previous studies have shown that while early aortic lesions were similar in the two areas, advanced aortic lesions were more severe in New Orleans, and the difference increased with advancing age. The present demonstration of variations in coronary atherosclerosis also indicates that the factors responsible for the development of fibrous plaques and hemorrhagic, ulcerated plaques with thrombosis are different from those which initiate the fatty streaks. Furthermore, the reaction of the coronary arteries to these factors appears to differ from that of the aorta.

THE DISTRIBUTION OF RENAL LIPIDS IN BENIGN AND MALIGNANT HYPERTENSION. Sidney P. Kent, University of Alabama Medical Center, Birmingham, Ala.

In searching for fat emboli by means of Sudan IV stains in kidney sections from 100 consecutive adult necropsies, an unusual distribution of lipid was noted in 3 of the patients. A number of the glomeruli contained a granular sudanophilic material which did not appear to be in the lumens of the capillaries primarily but lay in the endothelial cells and intercapillary spaces. Some of the glomeruli were almost completely filled with lipid. These glomeruli were not of decreased size, nor did they appear to be scarred. The tubules contained fatty casts, and a number of arterioles exhibited sudanophilia. On examining the clinical records, it was found that these 3 patients were considered to be classical instances of malignant hypertension.

To determine whether this distribution of lipid in the kidneys was characteristic of malignant hypertension, Sudan IV and oil red O preparations were made of the kidneys from 29 patients with clinical manifestations of malignant hypertension (diastolic blood pressure of 120 mm. of Hg or above, and eye ground changes which included hemorrhages and exudates). Renal sections from 35 cases of benign hypertension were also studied. In addition, sections of kidneys from 2 patients with embolic glomerulonephritis, 4 patients with acute glomerulonephritis, 6 with intracapillary glomerulosclerosis, and 3 nonhypertensive patients with renal failure were studied. While the distribution of lipids described above was not specific for malignant hypertension, it was sufficiently characteristic to be useful in differentiating malignant from benign hypertension at necropsy.

THE EFFECTS OF CORTISONE UPON AORTIC INTIMAL REPAIR IN THE HYPERCHOLESTEROLEMIC RABBIT. John T. Prior,* Robert F. Rohner, Frank A. Camp and Herman Rustad, State University of New York, Upstate Medical Center, Syracuse, N. Y.

This study is a continuation of previous investigations upon aortic injury and repair in normal and hypercholesterolemic rabbits. We previously demonstrated that repair following intimal trauma in normal rabbits produced a fibroelastic plaque about 35 days after injury. Lipid material and macrophages were never prominent, but calcification of the injured segment was noted. Similar trauma in hypercholesterolemic rabbits caused increased permeability to cholesterol in injured areas resulting in a fatty plaque histologically identical with the early human arterial lesions. Cholesterol, however, inhibited connective tissue repair so that counterparts of advanced human lesions were not seen.

In the present study, cortisone was administered to a series of normal and

hypercholesterolemic rabbits, in some of which the aortic intima had been previously traumatized. It was postulated that the known effects of cortisone upon mesenchymal structures might serve to add to the existing knowledge of the role of injury and repair in human atherosclerosis. Cortisone dosages ranged between 0.1 and 25 mg. per day over periods up to 3 months. The effects of cortisone upon 4 groups of animals were studied: (a) normal rabbits, (b) hypercholesterolemic rabbits, (c) rabbits with previous aortic injury, (d) hypercholesterolemic rabbits with aortic trauma. The results of the study revealed inhibitory effects of cortisone upon granulation tissue and a decreased number of macrophages in the injured segment. The latter observation may have special significance since fat deposition was absent within the injured area in the cortisone-cholesterol-trauma group. Spontaneous atherosclerosis was either absent or markedly inhibited in nontraumatized areas in the hypercholesterolemic group. The role of macrophages in the genesis of plaques is discussed, and some possible reasons for their inhibition in the cortisone-treated animals are advanced.

MUCOPOLYSACCHARIDES AND EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS. Ira Gore* and Richard Barr, Veterans Administration Hospital, West Roxbury, and Harvard Medical School, Boston, Mass.

Recent observations by several investigators have suggested that the initial change in atherosclerosis occurred in the ground substance of arterial intima and that the accumulation of lipid was a secondary phenomenon. To evaluate this thesis, 18 weanling rabbits were placed upon a cholesterol enriched, atherogenic diet. As controls, 6 similar rabbits were maintained on a standard diet. At intervals ranging from 22 to 132 days, the experimental animals were sacrificed in groups of 3 or 4, together with one of the controls. Histologic studies on formalin-fixed material included Alcian blue-PAS preparations for acid mucopolysaccharides, and stains for elastic and connective tissues. During the course of the experiment, blood samples were taken to permit serial determinations of serum cholesterol, mucoprotein, protein bound hexose, and hexosamine. The blood proteins, including lipoprotein, and glycoprotein were studied by paper electrophoresis. Augmented quantities of ground substance did not appear in the vessel wall until atheromatous lesions had been established. Accumulations accompanied proliferation of connective and elastic tissues and could be interpreted as part of the reaction to the primary deposition of intimal lipid. The local process in the vascular tree was not reflected in changes in the levels of circulating mucoprotein, hexosamine, or protein bound hexose.

A HISTOCHEMICAL STUDY OF THE POLYSACCHARIDES OF THE AORTA. Herbert Braunstein,* College of Medicine, University of Cincinnati, Cincinnati, Ohio.

The structure of the aorta was outlined by special histologic and histochemical means in a study of sections of ascending aortas secured at necropsy from 200 individuals, aged 16 to 97 years. Elastic plates were found to make up roughly 20 per cent of the bulk of the fixed, embedded aorta. Smooth muscle varied considerably in quantity, but generally constituted less than 5 per cent of this volume. Connective tissue varied in proportion from 15 to 30 per cent. A relatively constant proportion of the aortic wall, roughly of equal quantity, consisted of PAS positive material. A small amount of this material within smooth muscle cells was digestible by diastase, while the remaining extracellular portion was resistant to both diastase and hyaluronidase digestion. Both acetylation for 2 hours and bisulfite treatment destroyed the PAS positive staining. This material appeared to represent the reticulum fibrils and a portion of the collagen of the aorta.

The remainder of the wall of the aorta consisted largely of extracellular acid mucopolysaccharide. This substance was metachromatic with toluidine blue, with optimum staining at pH 6 and virtually none at pH 2. Sulfation tended to in-

crease metachromasia at lower pH levels. The material was stainable with Alcian blue, Alcian green, and Hale's colloidal iron stain, but was PAS negative when sequential staining techniques were employed. It was digestible by testicular but not by streptococcal hyaluronidase. An estimate of the quantity of this substance was made by means of a grading technique, using a scale of 1 to 4 plus. It is noted that there was considerable variation in the quantity present in different aortas. Accumulations were sometimes encountered in the absence of damage to elastica, but large deposits were always present in younger atheromatous plaques, and in areas of damage to elastica resulting from any cause, including syphilis and arteriosclerosis. There was a tendency for greater quantities to be present in the internal third of the wall of the aorta. No correlation of quantity with age was apparent.

It is concluded that much of the wall of the aorta is a loosely organized mass of acid mucopolysaccharide normally varying in quantity to a considerable degree from individual to individual. The mucopolysaccharide probably acts as a filler substance, tending to fill vacuums created by a variety of forms of damage to elements of the wall.

ANATOMIC FEATURES OF THROMBOSIS INDUCED IN RATS BY DIETARY MEANS ALONE.† R. M. O'Neal,* W. S. Hartroft* and W. A. Thomas,* Washington University School of Medicine, St. Louis, Mo.

Spontaneous arterial thrombi and associated renal and cardiac infarcts occurred regularly in a percentage of rats fed diets high in fat (40 per cent), especially butter. The basal diet also contained 0.3 per cent thiouracil, 5 per cent cholesterol and 2 per cent sodium cholate. Three separate experiments were performed, and similar results were obtained in each. Mortality among the rats was high. Arterial thrombi or infarcts could be demonstrated in approximately half of those surviving two months or longer. The percentage of rats with thrombi varied with the type of dietary fat given. Serum cholesterol levels were often above 2,000 mg. per hundred cc. of blood.

Lipidosis of the aortic valve ring was encountered frequently, but practically no other gross or microscopic lesions of the arterial walls could be seen except when special stains for lipid were applied. Diffuse and often extreme degrees of infiltration of arterial walls by fine fat droplets were common. Thrombi were found in various stages of organization but consistently contained large amounts of stainable fat. This association between fat and thrombi was conspicuous in the recent fibrin thrombi, as well as in the completely organized ones. The study of the lipid component of the lesions was facilitated by the use of Carbowax for embedding tissue.

CALCIFIC AND LIPID-CONTAINING VASCULAR LESIONS IN RATS WITH NEPHROTOXIC RENAL DISEASE. Donald B. Hackel* and Walter Heymann, Cuyahoga County Hospital, and Babies and Children's Hospital, Western Reserve University, Cleveland, Ohio.

Rabbit anti-rat-kidney serum was given intravenously to 51 Long-Evans rats, in a dosage sufficient to produce a chronic, progressive renal disease. The rats were fed a normal diet ("Friskies"). At the time of sacrifice, 1 to 12 months after the injection, blood chemistry studies were carried out on heart's blood, roentgenograms were made of the heart and aorta, and microscopic sections of kidney, heart, and aorta were prepared. The terminal blood chemistry studies gave evidence of renal failure, with marked azotemia and elevation of serum cholesterol and total lipid values. The serum calcium was normal or high in most cases, and

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the serum phosphorus was invariably high, with values between 9 and 32 mg. per hundred cc. of blood. The kidneys of all cases showed microscopic evidence of severe renal disease, with fibrosis of glomeruli and tubular degeneration. Thirty-four of the 51 rats exhibited calcified medial or fatty lesions of the aorta and coronary and renal arteries. The amount of fat in the intima or media bore no relation to the presence or intensity of medial calcification, nor had it any consistent relation to the degree of lipemia.

The hypothesis is advanced that chronic renal lesions resulting from the injection of anti-kidney serum and leading to renal failure, can result in secondary hyperparathyroidism and subsequent arterial calcification. Although lipid-containing arterial lesions often occurred in these animals, they seemed to bear no relationship to the areas of calcification, and no direct relation to the height of the blood lipid values.

OBSTRUCTIVE LESIONS OF THE RENAL ARTERY ASSOCIATED WITH REMEDIABLE HYPERTENSION. Lawrence J. McCormack,* John B. Hazard,* and Eugene F. Poutasse, The Cleveland Clinic, Cleveland, Ohio.

The recent recognition of segmental occlusive disease of the renal artery as a cause of remediable hypertension has resulted from improved renal angiography. The mechanism of the elevation of blood pressure may be similar to that produced experimentally by Goldblatt. Surgical exploration has provided specimens of renal arteries and of kidneys from patients in whom renal arterial disease was associated with reversible hypertension. It is our purpose to present the result of pathologic studies of 19 seriously diseased segments of renal arteries and of portions or all of 35 affected kidneys.

The pathologic observations in the renal arterial segments were separated into 4 categories on the basis of the apparent cause of the obstruction. The first group included 10 vessels that appeared to be affected by some phase of arteriosclerosis; 7 showed a partially occluding eccentric fibrous or fibrofatty plaque; 3, in addition to this plaque, showed a poststenotic dissecting aneurysm. In a second group, composed of 4 arteries, thrombosis was the basic disorder; in 3, no underlying cause was demonstrated, and in 1, a moderate degree of adventitial inflammation was present. A third group consisted of 4 vessels from 3 young patients, and showed an unusual form of fibromuscular hyperplasia of the arterial wall which produced marked segmental stenosis of the vessels. The fourth group included only 1 vessel, representing a segmental arterial lesion interpreted as mural scarring involving the full thickness of the arterial wall and producing marked luminal stenosis. Eight of the 35 renal specimens showed no significant lesions. Some degree of renal atrophy was the prominent feature in 24 specimens. The severity of this alteration ranged from minimal focal cortical scarring to a diffuse atrophy, involving the renal tubules but sparing the glomeruli. Several kidneys showed only a segmental area of atrophy, sharply demarcated from normal renal tissue. Total renal infarction did not occur in any case. Localized foci of chronic pyelonephritis were found in the 3 remaining specimens.

A brief comment upon clinical correlations and courses subsequent to operation will form a portion of the presentation.

CONGENITAL MITRAL ATRESIA. Gordon F. Vawter, The Children's Hospital and Harvard Medical School, Boston, Mass.

Nineteen instances of aplasia or atresia of the mitral valve were observed at necropsy at The Children's Hospital, Boston, between 1931 and 1957. These cases were reviewed in the context of accompanying malformations, especially of the cardiovascular system, although partial malrotation of the abdominal viscera was commonly found. Thirteen had relative deficiency of aortic outflow, representing 3 differing but apparently embryologically related configurations. In 7, the aortas

were transposed to the right ventricle (4 had complete atresia). The simplest of the configurations (6 cases) was accompanied by extracardiac anomalies, incompatible with long survival in 3. In 4 there was an associated deficiency of the pulmonary outflow with complex malformations, apparently similar to those described above. Both great vessels arose from the right ventricle in these cases. Associated congenital anomalies were also common in this group. "Corrected transposition" was noted in 2 cases and was least easily fitted into a unified concept of the embryogenesis of the mitral lesion.

The prognosis for survival appeared greater in the latter 2 groups of malformations. Only 3 instances of unusual return of pulmonary venous blood to the right atrium were recognized.

THE AGING HEART. I. ENDOCARDIUM. James B. McMillan and Maurice Lev,* Mount Sinai Hospital, Miami Beach, Fla., University of Miami School of Medicine, Coral Gables, Fla., Hektoen Institute, and Northwestern University Medical School, Chicago, Ill.

A gross and microscopic study was made of the changes associated with age in the endocardium in 100 normal human hearts. Grossly, a progressive gray-white thickening developed within the first decade. This was focal in the right atrium and both ventricles, and diffuse in the left atrium. With advancing age, the circumscribed areas became more prominent and geographic in pattern. In the left atrium similar plaques became superimposed upon the areas of diffuse thickening. Histologically, the focal and diffuse thickening reflected endocardial hypertrophy, i.e., a proliferation of the elastic, collagen, and muscle fibers of the endocardium. In foci of hypertrophy, degenerative changes or endocardial sclerosis occurred consisting of: (a) fragmentation and disruption of elastic fibers; (b) vacuolization and atrophy of smooth muscle cells; (c) patchy staining of ground substance; (d) irregular staining of basement membranes; (e) slight lipid infiltration; (f) collagen replacement with eventual hyaline sclerosis; and (g) slight focal calcification. The subendocardium also showed changes with advancing age.

The observations are discussed in the light of laws governing tension and pressure in vessels. Endocardial hypertrophy is considered to result from two distinct though related mechanisms, focal endocardial hypertrophy from "flow impact," and diffuse endocardial hypertrophy from increased intramural tension. Similarly, endocardial sclerosis results from intramural tension, "flow impact," and hydrostatic pressure. It is considered that endocardial hypertrophy, endophlebohypertrophy, and endarteriohypertrophy are similar, and related to "flow impact," whereas endocardial sclerosis, endophlebosclerosis, and endarteriosclerosis are related to hydrostatic pressure and "flow impact." Thus, arteries reveal severe endarteriosclerosis and relatively less endarteriohypertrophy, veins exhibit marked endophlebohypertrophy and relatively less sclerosis, and the endocardium has the lowest degree of sclerosis in relation to hypertrophy.

RADIOLOGIC STUDY OF THE HEART IN GUINEA PIGS LIVING AT SIMULATED HIGH ALTITUDE. Enrique Valdivia and LeRoy L. Appel, University of Wisconsin Medical School, Madison, Wisc.

Dilatation followed by hypertrophy of the right ventricle was demonstrated anatomically in guinea pigs living for prolonged intervals at a simulated high altitude of 18,000 feet in a low pressure chamber. During these experiments, the chamber was opened periodically for cleaning and feeding. Twenty controls and 25 experimental guinea pigs were studied. Radiologic examination consisted of a postero-anterior exposure to demonstrate the cardiac shadow. Intracardiac injection of radiopaque substance demonstrated the individual chambers. Between the 10th and 20th days of exposure to simulated high altitude, the cardiac shadow was temporarily enlarged. This change was better demonstrated by the injection

of opaque material whereby enlargement of the right ventricular chamber was observed as early as the first week. The pulmonary arteries and their branches also appeared greatly enlarged after one week of exposure. Dilatation of the right ventricle subsided progressively, and it was not seen after the sixth week. The significance of these observations as evidence for pulmonary hypertension due to chronic hypoxia will be discussed.

INFANTILE HYPERPLASTIC CARDIOMEGALY. B. Black-Schaffer* and M. E. Turner, University of Cincinnati College of Medicine, Cincinnati, Ohio, and North Carolina State College, Raleigh, N. C.

Cardiomegaly in infancy is ordinarily dismissed as a result of hypertrophy. Twenty normal hearts varying in weight between 70 and 277 gm. and 8 instances of cardiomegaly, 4 with endocardial fibroelastosis, were investigated by means of nuclear counts of the muscle fibers. From this study it was concluded that the instances of idiopathic cardiomegaly with fibroelastosis represented examples of true muscle hyperplasia in contrast to the hypertrophy associated with the cardiomegaly of chronic rheumatic valvular disease and hypertension of chronic glomerulonephritis and pyelonephritis.

It was demonstrated that the surface area and the length and volume relationships of the hyperplastic fibers, in addition to their numbers, resulted in a less efficient organ, thus explaining the initial postnatal dilatation of the heart with the inception of a vicious circle culminating in cardiomegaly with or without endocardial fibroelastosis.

ALVEOLAR PROTEINOSIS. Samuel H. Rosen,* Benjamin Castleman,* and Averill A. Liebow,* the Armed Forces Institute of Pathology, Washington, D. C., Massachusetts General Hospital, Boston, Mass., and Yale University School of Medicine, New Haven, Conn.

Twenty-six instances of a pulmonary disorder resembling, but we believe different from, *Pneumocystis carinii* infection, were encountered by the writers within the past 5 years. The process was characterized by the filling of pulmonary alveoli by a PAS-positive proteinaceous material which was rich in lipids. This material appeared to be produced by the lining cells which desquamated into the lumen, ultimately becoming necrotic and yielding granules and laminated cystlike bodies to the alveolar content. Reaction to this material was minimal.

Clinically, the disease was on occasion initiated by symptoms indicating pneumonitis. The most common and prominent complaint was dyspnea, usually accompanied by a cough that was in some instances productive of yellow sputum. Weight loss, fatiguability, and chest pain occurred in a small proportion of the patients. Cyanosis was observed when the extent of involvement was most severe. The lung fields were usually normal to percussion and auscultation. Roentgenographically, however, the appearance was that of a diffuse perihilar, radiating, feathery, or vaguely nodular soft density resembling that seen in severe pulmonary edema. The clue to the diagnosis was the inappropriateness of the symptomatology in the face of the roentgenographic appearance. The occupations of the patients were varied. The only clue of possible exposure to an injurious inhalant occurred in 4 of the 26 patients, who worked in lumber yards; one patient was a carpenter, and two were electricians. Most of the patients were young adults, 20 to 40 years of age, but there was one child aged 2½ years.

The prognosis varied. Four patients had a relatively stationary course, one for 4½ years without change in the roentgen films. At least 2 were showing signs of recovery. Seven died, and in 3 of these, fungi of diverse types had produced lesions superimposed upon those of the underlying disease. Because of existing ignorance of the cause of this condition, the designation "alveolar proteinosis" has been tentatively chosen.

THE HISTOLOGY OF "FARMERS' LUNG." Robert S. Totten* and Thomas J. Moran,* Presbyterian and Woman's Hospitals, and University of Pittsburgh School of Medicine, Pittsburgh, Pa.

The disease known as "farmers' lung" in Great Britain and "threshers' lung" in Sweden is a distinct clinical entity characterized by severe dyspnea accompanied by varying degrees of fever, chills, cyanosis, and prostration. It is closely related to the inhalation of "moldy" hay or silage although the ultimate agent is not known. Because of the self-limited nature of the condition, little has been written concerning the histologic changes in the affected lungs. Two farmers from Western Pennsylvania who suffered from the disease were recently observed, and lung biopsy specimens provided an opportunity to study the histologic lesions. A diffuse interstitial pneumonitis with the formation of miliary granulomas was the most striking alteration. A similar intramural reaction occurred in bronchioles, and a focally distributed obliterating bronchiolitis was observed. Microscopic emphysema was seen in both cases, and in one there was interstitial fibrosis.

PULMONARY CHANGES IN THE SO-CALLED "COLLAGEN DISEASES." Joseph C. Sieracki, Robert C. Horn Jr.* and William R. Eyler, Henry Ford Hospital, Detroit, Mich.

The clinical data and pathologic material from all cases of so-called "collagen disease" seen at the Henry Ford Hospital during a 4 year period have been analyzed with particular attention to pulmonary lesions. The conditions studied included lupus erythematosus, scleroderma, periarteritis nodosa, necrotizing angiitis, and selected examples of rheumatic disease. In many instances the appearance of the chest roentgenograms was sufficiently specific to permit the radiologist to suggest or confirm a diagnosis of "collagen disease". However, the pathologic lesions were quite variable. In scleroderma, rheumatic fever, and Wegener's granulomatosis, reasonably specific pulmonary lesions were found. Although in the other diseases definite and irreversible alterations commonly occurred, these were variable, and susceptible to many limitations of interpretation. Selected examples of each group will be presented.

PULMONARY ASPERGILLOSIS ASSOCIATED WITH CORTISONE AND ANTIBIOTIC ADMINISTRATION; HUMAN AND EXPERIMENTAL STUDIES. Herschel Sidransky and Lorraine Friedman, Tulane University School of Medicine, New Orleans, La.

An increasing number of patients with secondary fungal infection complicating other disease have been reported in recent years. At Charity Hospital in New Orleans 15 cases of secondary fungal infection of the lung have been observed among the routine necropsies performed in the Tulane Service from 1955 through 1957. Five of the patients had pulmonary aspergillosis, and each of these had received therapy with cortisone and broad-spectrum antibiotic agents. *Aspergillus flavus* was cultured in 3 cases. The gross and microscopic findings of the pulmonary lesions will be described.

To investigate the possible relationship between pulmonary aspergillosis and treatment with cortisone and broad-spectrum antibiotic agents, mice were injected with cortisone and penicillin 2 days before exposure to clouds of spores of *Aspergillus flavus*. Tetracycline was given in water bottles throughout the experiments. Control groups (1) exposed to fungus spores but receiving no cortisone or antibiotic therapy and (2) receiving cortisone and antibiotic agents but no exposure to spores, were also studied. Deaths occurred among the experimental mice in from 1 to 14 days depending upon the dosage of spores; no deaths occurred in the control groups. The pulmonary lesions in the experimental mice resembled those seen in the human cases and consisted of bronchopneumonia accompanied by hyphal invasion of blood vessels. No lesions were found in the control animals. These results indicate that the administration of cortisone and broad-spectrum

antibiotic agents increases the susceptibility of mice to pulmonary aspergillosis. It is probable that patients treated in similar fashion may likewise be more susceptible to infection with air-borne aspergilli.

CLINICAL AND MORPHOLOGIC FINDINGS IN 30 FATAL CASES OF INFECTIOUS MONONUCLEOSIS. Robert J. Lukes, and Franklin H. Cox, Armed Forces Instititute of Pathology, Washington, D. C.

Although infectious mononucleosis is a fairly common disease, morphologic observations in relatively few fatal cases have been reported. Necropsy material from 30 cases available for study at the Armed Forces Institute of Pathology exhibited widespread proliferation in lymphoid tissue of a characteristic mononuclear cell. This occurred principally in the spleen, lymph nodes, bone marrow and liver, but was also occasionally prominent in the gastrointestinal tract and nasopharynx. Small collections of mononuclear cells could be found in any of the viscera and also in nerve roots and the leptomeninges.

The 3 major causes of death in this series were: spontaneous rupture of the spleen (13 cases); Guillain-Barré syndrome (6 cases); and hemorrhage from the nasopharynx or gastrointestinal tract (4 cases). The 7 other patients died from a variety of causes, the most common being secondary infection.

The proliferation of mononuclear cells was observed in its most severe form in the spleen, where it obscured the architectural features and caused infiltration of the capsule and trabeculae. These changes were most advanced in the cases with spontaneous rupture. Involvement of the nervous system was most pronounced in cases in which death was the result of the Guillain-Barré syndrome. It was manifested by degeneration of the myelin sheaths of the nerve roots and focal perivascular infiltration of the leptomeninges and peripheral nerves by mononuclear cells. Similar infiltration was noted in a few cases in which evidence of the Guillain-Barré syndrome was lacking. In a few instances, dense collections of mononuclear cells were observed in both the nasopharynx and gastrointestinal tract, usually in sites of pre-existing lymphoid tissue. Ulceration of the overlying mucosa was common and probably represented an important factor in the severe hemorrhage observed clinically in 4 cases. Despite considerable variation in the clinical manifestations, its duration and the complications which led to death, the characteristic mononuclear cell proliferation was constant in distribution and varied only in degree.

HORMONAL EFFECTS ON A SARCOID-LIKE RESPONSE WITH SCHAUMANN BODIES AND AMYLOID IN GOLDEN HAMSTERS INFECTED WITH PHOTOCHROMOGENIC MYCOBACTERIA. J. K. Frenkel,* University of Kansas Medical Center, Kansas City, Kans.

The granulomatous response in the following groups of hamsters infected with mycobacteria was compared (days of mean survival time in parentheses): intact males (183) and females (118); radiothyroidectomized males (194) and females (136); males treated with zinc-corticotrophin, 2 units daily, begun 130 days after the initiation of infection (171); adrenalectomized males maintained on deoxycorticosterone trimethylacetate, 1.25 mg. monthly (85); males treated with cortisone acetate, 2.5 mg. weekly (57); males on a diet containing 0.4 per cent propylthiouracil (163); and males on a diet containing 1 to 2 per cent desiccated thyroid powder (37), the larger dose being toxic.

Four months after infection, organisms in lesions were most numerous following cortisone and least after thyroid medication, with larger numbers in females than in males. Mycobacteria grew intracellularly in epithelioid and giant cells which replaced lymphoid tissue and bone marrow. Epithelioid cells were larger showing a granulated cytoplasm in hamsters treated with ACTH, cortisone, and thyroid. Cortisone treatment markedly limited the number of epithelioid cells

formed. Necrosis was absent, except following ACTH therapy. Occasionally, innumerable mycobacteria grew within adrenocortical cells which became necrotic. Giant cells were absent after cortisone treatment, but were common in the lesions of ACTH-treated and thyroidectomized hamsters. Laminated Schaumann bodies, containing calcium and iron, and forming around acid-fast bacilli were numerous in most groups, especially following thyroidectomy; they were disproportionately rare in animals receiving cortisone and the thyroid supplement, compared to the number of mycobacteria present. Similar bodies were found in hamsters with histoplasmosis, the fungus forming the core of the laminated body. Para-amyloid deposits were common in the spleen, liver, kidney, adrenal, and thyroid gland. Thyroid medication accentuated, and thyroidectomy and cortisone inhibited the formation of amyloid.

THE PATHOLOGY OF FATAL COXSACKIE INFECTION IN THE NEWBORN. Kurt Benirschke,* Sidney Kibrick, and John M. Craig,* Boston Lying-in Hospital, and The Children's Hospital and Harvard Medical School, Boston, Mass.

Three infants dying in the first 10 days of life, in whom Coxsackie B3 and B4 viruses were isolated at necropsy from diseased tissues, were found to have widespread inflammatory and destructive lesions. Common to all 3 patients were encephalitis and marked myocarditis with signs of congestive heart failure. In 2 cases there was liver necrosis. In one instance, hepatic and adrenal necrosis apparently had antedated birth. Other lesions found were fat necrosis, pancreatitis, and inflammation of smooth muscle coats. In all cases upper respiratory infections were present in the mother or other members of the family, and in some there was biologic and serologic evidence of infection with agents identitical to those encountered in the infants. A comparison of the pathologic features with those encountered in experimentally induced infections in newborn mice will be made.

COXSACKIE INFECTION IN INFANTS AND CHILDREN: FOCAL AND DISSEMINATED; FATAL AND NONFATAL. W. A. Newton, Jr., and D. M. Hosier, College of Medicine, Ohio State University, Columbus, Ohio.

Coxsackie viruses are considered to be among the most prevalent of recognized viruses. They are known to cause herpangina, epidemic pleurodynia, and aseptic meningitis, but until recently have not been thought to cause death. To date, 5 reports have described fatal myocarditis in newborn infants caused by type B3 or B4 Coxsackie virus. Four of these reports originated abroad in The Netherlands and in South Africa and described epidemics of fatal myocarditis occurring in newborn infants during epidemics of pleurodynia or aseptic meningitis in the population at large.

We are reporting the isolation of type B4 Coxsackie virus at necropsy from the myocardium, liver, brain, and kidney of a 14-day-old infant, dying of sepsis of unknown cause, and from the myocardium of an 8½-week-old child, who died in heart failure. Type B4 Coxsackie virus was also isolated from the stool of a 5-year-old boy, who developed severe myocarditis two weeks after an influenza-like syndrome. He recovered completely and showed a rise of homologous serum neutralizing antibodies. From this evidence it appears that type B4 Coxsackie virus can cause death in early life, either by a localized infection of the myocardium, or by a generalized process affecting the myocardium, central nervous system, liver, and adrenal gland. The recovery of the virus in the stool coupled with a rising titer of homologous neutralizing antibodies strongly suggests that type B4 Coxsackie virus may cause nonfatal myocarditis.

We have reviewed our necropsy case material of infants and children in which focal or diffuse myocarditis was found. A striking seasonal incidence was noted. Seven of 10 of the children died during July, August, and early September. This period approximates the peak of seasonal incidence of Coxsackie virus infection.

Five of the 10 children showed either focal hepatic necrosis or encephalomyelitis, or both, in addition to the myocarditis. This distribution of lesions was similar to that observed in the newborn infant whose tissues yielded type B4 Coxsackie virus. The seasonal incidence and the distribution of the extracardiac lesions suggest that Coxsackie viruses may be the causative agent of some cases of idiopathic myocarditis.

CHANGES IN THE FINE STRUCTURE OF CEREBELLAR NEURONS FOLLOWING IONIZING RADIATION. F. Stephen Vogel,* Cornell University Medical College New York, N. Y.

Dogs and rabbits received radiation to the cerebellum with 15,000 r. of gamma rays from a CO^{60} source. Electron microscopy disclosed that the earliest recognizable cytologic changes, occurring in the granule cells within 4 hours, were regularly characterized by contraction of the nucleus, clumping of the intranuclear granules with margination toward the nuclear membrane, serration of the outer nuclear membrane with occasional small bleb formation, expansion of the cytoplasm and swelling of individual mitochrondria with central rarefaction and fragmentation of cristae. Further contraction of the nucleus, to approximately one-half its normal size, brought about very close aggregation of the nuclear granules but without coalescence. The nuclear membranes became markedly redundant and folded, and further expansion of the cytoplasm occurred with wide dispersion of the cytoplasmic constituents. Disintegration of the nuclear membranes with death of cells was rare in rabbits but conspicuous in dogs. With recovery in rabbits, after 72 hours, the normal cellular structure was reconstituted.

The observations provided evidence that nuclear contraction and hyperchromatism (pyknosis) induced in the cerebellar granule cells by radiation, was associated with a rapid profusion of electron-lucent nuclear-sap from the karyoplasm into the cytoplasm. This cytologic alteration was clearly transitory in rabbits where fragmentation of the nuclear membranes rarely occurred. In dogs, it was more often accompanied by disintegration of nuclear membranes and cellular death.

EXPERIMENTAL STUDIES OF THE *in Vivo* RELATIONSHIPS OF THE PROPERDIN SYSTEM TO RESISTANCE TO INFECTION. Oscar A. Ross* Western Reserve University, Cleveland, Ohio.

The original description of the properdin system and the discovery that its effects were not due to specific antibodies prompted a study of host resistance to experimental infections in mice. These studies showed that the intraperitoneal injection of large numbers of *E. coli* into mice resulted in a marked fall in serum properdin level and death of the mice. The parenteral injection of zymosan resulted in an initial fall followed by a sustained rise in serum properdin levels accompanied by increased mouse resistance to infection with *K. pneumoniae*. Increased nonspecific resistance to infection with *K. pneumoniae* persisted in mice for periods up to 3 months after a single injection of zymosan. The resistance persisted despite the return to normal of serum properdin levels. Purified properdin administered intravenously into mice was rapidly destroyed in 2 to 24 hours. However, purified properdin (human) injected intravenously into mice 4 hours before or 2 hours following the intraperitoneal inoculation with *K. pneumoniae* resulted in marked protection of the mice.

CONDUCTION TISSUE STUDIES IN HUMAN AND CANINE HEARTS. George Lumb, R. S. Shacklett and W. A. Dawkins, University of Tennessee, and City of Memphis Hospitals, Memphis, Tenn.

Two hundred human hearts procured at necropsy from patients of varying ages, with and without evidence of cardiac disease, have been studied to identify

the conduction mechanism and its blood supply. Correlative studies have been performed in dogs. The main source of blood supply to the conduction mechanism in normal canine hearts is a posterior terminal branch of the left circumflex artery passing anteriorly across the floor of the right atrium to end in a capillary network near the root of the aorta; and an anterior vessel arising at the bifurcation of the left coronary artery and passing posteriorly to give branches into the moderator band which terminate in a free anastomosis with the posterior vessel already described.

Thoracotomies were performed through the fourth interspace, and vascular occlusion was effected. Oscillographic and electrocardiographic records were obtained at suitable intervals. Results thus far with 40 dogs indicate that infarction of large muscle areas does not necessarily produce striking electrocardiographic changes unless the conduction mechanism is involved. On the other hand, small solitary infarcts produced by selective occlusion of the blood supply which affects the conduction mechanism are associated with dramatic electrocardiographic alterations. This study is the first stage of a series of experiments intended to identify the anatomic site of the conduction tissue in dogs, its blood supply, and the amount of damage which can be tolerated. Results may be correlated with the sequelae of surgical manipulation of the interventricular septum in human subjects. Certain pathologic lesions affecting the conduction mechanism in human hearts will be demonstrated and correlated with electrocardiographic features.

INFLUENCE OF SUBCUTANEOUSLY ADMINISTERED BOVINE GLOBULIN ON THE RESPONSE OF RABBITS TO INTRAVENOUS GRAM-NEGATIVE ENDOTOXIN OR LIQUOID. Peter E. Fehr and Joel G. Brunson,* University of Minnesota Medical School, Minneapolis, Minn.

A daily subcutaneous injection of 0.5 ml. of a 10 per cent solution of bovine gamma globulin was administered to hybrid albino rabbits for 6 days. Seventy-two hours after the last injection of globulin, the animals received a single intravenous injection of endotoxin derived from gram-negative organisms or sodium polyanetholsulfonate (Liquoid). Those which did not die following the administration of the toxin or Liquoid were sacrificed 48 hours later. Fibrinoid lesions in the heart, lung, spleen, liver, and kidney were observed in 70 (85 per cent) of the animals given endotoxin and in 39 (61 per cent) of those receiving Liquoid. The coronary arterial lesions were associated with an inflammatory reaction which resembled that observed in polyarteritis. In the kidney, fibrinoid was deposited in a focal manner in scattered glomeruli and in the media of the large renal arteries. There was also pronounced glomerular endothelial proliferation which resembled the lesions of acute proliferative glomerulonephritis.

Serial paper electrophoretic studies of serum proteins were performed in 77 animals. These showed a reversal of the albumin-globulin ratio due to an increased amount of globulin and the presence of a para-beta globulin which migrated between the beta and gamma globulin fractions. A continued increase in the latter fraction was observed in the animals given Liquoid. Of those which received endotoxin, this fraction increased only in those rabbits which developed fibrinoid lesions. In control animals given only globulin, and in those which failed to develop fibrinoid lesions after endotoxin administration, a smaller amount of para-beta globulin was detected.

NATURALLY OCCURRING LYMPH NODE AND STROMAL REACTIVITY OF PROGNOSTIC SIGNIFICANCE IN HUMAN CANCER. Maurice M. Black* and Francis D. Speer, New York Medical College, Flower and Fifth Avenue Hospitals, New York, N. Y.

Variability in the behavior of different patients with the same types of cancer

has led to the concept of host resistance. The mechanism of such postulated resistance has not as yet been elucidated, and numerous immunologic studies have failed to demonstrate the existence of specific cancer antibodies. The present investigation was undertaken to obtain data bearing on the occurrence and biologic significance of reactive changes in the stroma of human cancer and the related regional lymph nodes. This report presents data obtained from more than 2,000 patients with cancer. Follow-up data, to death or at least for 5 years postoperatively, were available in approximately 1,000 cases.

It was shown that: (a) there appeared to be a relationship between biologic behavior and the occurrence of sinus histiocytic hyperplasia of the regional lymph nodes or lymphoid infiltrations in the primary tumor; (b) these structural features were encountered with varying frequency among different types of cancer; (c) cancer tissue did not seem to evoke lymph node reactivity of the hyperimmune type. The observations will be discussed in terms of the biologic phenomena involved and the need for further study of naturally occurring factors in host resistance to cancer.

FORMATION OF MYELIN FORMS FROM BRAIN TISSUE BY MEANS OF SURFACE ACTIVE SUBSTANCES. William W. Ayres, Armed Forces Institute of Pathology, Washington, D. C.

Dioctyl sodium sulfosuccinate, a synthetic detergent, was emulsified in human, dog, guinea pig, and cat brain tissue in concentrations of 2 to 17 per cent. A portion of each emulsion was placed on a slide, covered with a coverslip, sealed, and observed over a period of days with bright field and polarizing microscopes. Myelin forms "grew" in the preparations within 24 hours. The myelin forms were round, oval or elongated structures with highly refractile walls, which formed intricate tangled masses. Under the polarizing microscope they were anisotropic and optically positive. Some of the myelin forms were spiraled, which suggests that the structures described by Steiner as *Spirochaeta myeloptera* in lesions of multiple sclerosis may actually be myelin forms.

The emulsifying surfactant in certain concentrations was also anisotropic but could be distinguished from myelin forms by its shape and the lesser degree of anisotropy. The surfactant had the ability to control the direction of "growth" of myelin forms, indicating orientation of molecular structure by 2 liquid crystals. Cholesterol crystals and tyrosine crystals, indicative of lipoprotein breakdown, developed in the surfactant-brain emulsions. Cationic, nonionic and anionic surfactants and cobra venom caused formation of myelin forms.

Myelin forms similar to those observed in surfactant-brain emulsions were demonstrated in frozen sections of lesions in acute multiple sclerosis.

Speculations regarding the mechanism of formation of the myelin forms *in vitro* (and possibly *in vivo*) are as follows: (1) Surface active substances in contact with brain tissue split lipoprotein. (2) Lipids, particularly lecithin, in the presence of water develop into myelin forms by orientation of the hydrophilic portion of the lecithin molecules to water. Lecithin is heteropolar and also surface active. Thus a demyelinating process once initiated may be self-propagating. Still to be investigated is whether the splitting of lipoprotein by surface active substances is mediated through an enzymatic process. This is particularly pertinent, since bile salts, which are surface active, act as coenzymes for lipase. It is possible that in "allergic" encephalomyelitis a release of surface active substance during antigen-antibody reaction may initiate the demyelinating process. In this regard, Zunz demonstrated that the surface tension of plasma and serum was lowered in dogs subjected to anaphylactic shock and I have demonstrated formation of Charcot-Leyden crystals, common in allergic tissue reactions, from eosinophils *in vitro* by means of surface active substances. In a discussion of surface

tension phenomena in relation to demyelination one must mention the work of Weil who demonstrated that bile salts and other surface active substances caused demyelination *in vitro*. He has long believed that a surface active substance derived from the liver is operative in multiple sclerosis.

THE ULTRAVIOLET FLYING SPOT TELEVISION MICROSCOPE. P. O. Montgomery* and W. A. Bonner, The University of Texas Southwestern Medical School, Dallas, Texas.

This is a short presentation of the electronic and optical equipment necessary to obtain time lapse motion pictures of the ultraviolet absorption images of undamaged living cells. The salient features of the technical operation of the equipment are described. The unique features of the equipment are: (1) The light source consists of an ultraviolet cathode ray scanner tube. (2) The microscope is used to minify the image of the line raster of the light source so as to make it scan the living cell specimen. (3) Image conversion is obtained by photomultiplier tube modulation of a television screen, thus allowing for a 10 fold reduction in light over the photographic plate method. (4) Time lapse motion picture synchrony is obtained by activation of the camera mechanism by the scanner tube blanking pulse. (5) Intense focal irradiation of the specimen may be obtained for any area down to 1μ in size. (6) Radiation dosage may be varied by 3 means: (a) variation in the intensity of the emission of the ultraviolet scanner tube; (b) variation of the sweep cycle of the raster of the ultraviolet scanner tube from 1/30 of a second to a 10 second frame speed; (c) pulsation of the raster for any number of frames followed by an interval of any number of frames.

STUDIES IN HISTOLOGY OF THE DUODENUM. Raymond Yesner,* Raphael D. Schwartz and Howard M. Spiro, Veterans Administration Hospital, West Haven, Conn.

Pieces of small intestine mucosa were obtained by means of the Shiner tube in 18 patients. Histologic study revealed excellent preservation of tissue, as contrasted with the usual autolyzed necropsy material.

In 3 cases, during subtotal gastrectomy biopsy specimens of the jejunum were obtained. The mucosa showed a normal, slender, filiform pattern. The remaining 15 biopsy fragments were duodenal in origin. Five of these were procured from patients with achylia gastrica, pernicious anemia, "irritable bowel syndrome," regional enteritis, and ulcerative colitis. These showed tall, slender villi, moderate numbers of eosinophils and plasma cells, and were considered normal. Specimens from 3 patients with lesions in adjacent tissue, i.e., antral gastritis, gastric carcinoma, and chronic relapsing pancreatitis, and from 5 patients with chronic diarrhea, showed varying and sometimes marked alterations in villous pattern, and increased inflammatory cell infiltration. These changes, though nonspecific, were considered to be abnormal. One case of classical sprue yielded a duodenal specimen with pronounced eosinophil and plasma cell infiltration, complete flattening of villi, and marked dilatation of Brunner's glands. One case of Whipple's disease yielded a diagnostic duodenal specimen, in which Brunner's glands again were greatly dilated.

THE ROLE OF THE VERTEBRAL VENOUS PLEXUS IN THE DISSEMINATION OF LABELED BLOOD CLOTS. Joseph P. Wack and John P. Wyatt,* St. Louis University School of Medicine, St. Louis, Mo.

The anatomic distribution and functional role of the vertebral venous plexus in the dissemination of embolic material was studied, utilizing radioactive emboli. A permanent vertebral venous plexus was produced by ligation and section of the inferior vena cava and azygos veins in dogs. Urokon angiography and viny-

lite casts confirmed the establishment of the plexus. Canine blood in which radioactive iron^{59} had previously been incorporated *in vivo*, was allowed to clot. The clot was dehydrated and emboli of graded size were prepared. In a series of control and test dogs a measured radioactive embolic "dose" was injected via the femoral vein. The terminal lodgment sites of these emboli were determined by radioactive scanning methods. Their intrinsic distribution within the pulmonary lobes and lobules was delineated by macrosection autoradiographic technics. The partitional distribution of labeled emboli entering into the caval system via the vertebral plexus was plotted. A preliminary study on the influence of the animal's position upon the end sites of lodgment was undertaken with the animals in planographic, supine, dextral, and sinistral planes, as well as with cephalic and caudal suspension.

The passage of labeled emboli as a means of charting pathways of dissemination, the filtering capabilities of Batson's plexus in relation to embolic "dose," the circulatory flow and the influence of position on lodgment sites have been studied.

Acanthamoeba (CASTELLANI): EXPERIMENTAL INFECTION IN LABORATORY ANIMALS. C. G. Culbertson,* J. W. Smith, H. K. Cohen and J. R. Minner, The Lilly Research Laboratories, Indianapolis, Ind.

This ameba, similar to if not identical with that first described by Castellani in 1930 was parasitic upon a culture of yeast. It has since been reported in cultures of monkey kidney cells upon two occasions. The second instance occurred in our laboratory. The amebas were detected in the culture fluid after they had been observed in lesions of the central nervous system, following intracerebral inoculation. The organism was found to cause the death of both mice and monkeys on intracerebral inoculation. Intranasal instillation after light anesthesia resulted in extensive invasion of the nasal cavity, brain, lung, and kidney. Since no bacterial or viral agent was demonstrated in association in this instance, the ameba appeared to be pathogenic under the conditions of the experiment. This report will describe details of the experiment and the lesions produced.

THE HISTOLOGY OF THE MACROGLOBULINEMIA OF WALDENSTRÖM. Thomas F. Dutcher and John Fahey, Pathologic Anatomy Branch and General Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.

In 1944 Waldenström described a clinical syndrome characterized by the presence of an abnormal quantity of serum globulins of high molecular weight. He named this syndrome "macroglobulinemia." Since 1944 there have been reports of more than 60 patients in whom ultracentrifugal analysis has proved the presence of macroglobulinemia. Despite the numerous reports of patients with this condition, very few detailed descriptions of the histologic findings have appeared, and no necropsy reports have been published in the American literature.

The histologic features in 2 necropsied patients who had macroglobulinemia were characterized by a proliferation of abnormal cells of the reticuloendothelial system with an apparent differentiation along lymphocytic or plasmocytic lines. The organs most prominently affected were the bone marrow, spleen, and lymph node. Clinically and histologically the lesions were suggestive of some type of malignant lymphoma. An interesting feature was the occurrence of PAS-positive material within the nuclei of many of the abnormal cells. With numerous staining techniques, the intranuclear material and the intravascular plasma were found to stain identically. The histologic findings in these 2 patients will be compared with those in 10 detailed necropsy reports in the European literature.

THE RELATION OF NEURONAL CYTOPLASMIC BODIES TO PARKINSONIAN STATES. Lewis E. Lipkin, the Isaac Albert Research Institute of the Jewish Chronic Disease

Hospital, and State University of New York, College of Medicine, Brooklyn, N.Y.

A study of 53 necropsied patients suffering clinically from the parkinsonian state and 206 consecutive control patients (106 of which were matched with the parkinsonian group as to age, sex and race) was undertaken to determine the frequency, topographic distribution, histochemical and morphologic characteristics of the cytoplasmic structures which have on occasion been considered specific for idiopathic Parkinson's disease (paralysis agitans). These nongranular, polychromatrophilic cytoplasmic bodies in ganglion cells, first described by Lewy in 1912, have not been widely studied. They were found in ganglion cells in 75 per cent of the 53 patients with the parkinsonian state. Twenty-nine of these patients had true paralysis agitans, 9 were classified in the postencephalitic group, and 15 were not differentiated on clinical grounds into either group. Cytoplasmic bodies were found in 86 per cent, 44 per cent, and 74 per cent of each group respectively. They were also found in 5 per cent of the control cases, including those with and those without clinical evidences of neurologic disorder.

The bodies were found most frequently in the pigmented cells of the *substantia nigra,* but were encountered with less frequency in the *locus caeruleus* and other brain stem nuclei. Rarely, they could be demonstrated in the cerebral cortex, basal ganglia, and spinal cord. Histochemically, they appeared to be of protein nature; neither fat nor complex carbohydrate components were demonstrable. The cytoplasmic bodies were encountered at all stages of the disease. Observations indicated that they pursued a progressive evolution which could be correlated with their duration, and that they probably represented a nonspecific cytoplasmic alteration of an abiotrophic nature.

SENILE CARDIAC AMYLOIDOSIS. Leopold Buerger and Herbert Braunstein,* College of Medicine, University of Cincinnati and the Cincinnati General Hospital, Cincinnati, Ohio.

Senile cardiac amyloidosis is a variety of amyloid disease largely restricted to the heart and occurring almost exclusively in elderly individuals. This study was instituted in order to investigate statistically whether an apparent increase in incidence in recent years was real. Accordingly, sections of the heart from all patients over the age of 55 years necropsied at Cincinnati General Hospital during the years 1941 to 1944 (986) and a similar group during 1953 to 1956 (1,325) were reviewed. In the first test period, 12 cases of cardiac amyloidosis (1.2 per cent) were discovered; in the second, 30 cases (2.2 per cent) were found. When broken down by decades, it was found that the change in incidence was due almost entirely to an increased incidence of this disease in patients over 90 years of age, with no cases in 10 necropsies during 1941 to 1944, and 8 cases in 33 necropsies during 1953 to 1956. Sex, race and other factors were also analyzed. Typical histologic and histochemical features were demonstrated.

Pathologically, in only 1 of the 42 cases was there significant amyloidosis of the viscera other than the heart. The degree of cardiac involvement itself was variable. The amount of amyloid present was estimated histologically, and only 12 cases revealed major cardiac involvement (20 to 50 per cent replacement of the myocardium). Of these, 8 (67 per cent) manifested cardiac failure due in whole or in part to the amyloidosis. In 2 others, sudden or unexpected death had occurred. In the remaining 30 cases, there were only 3 in which the amyloidosis was thought to have contributed to the patient's death. Cardiomegaly does not appear to be a prominent feature of this disease unless some other factor such as hypertension, coronary arteriosclerosis with myocardial damage, or prolonged congestive cardiac failure is also present. There was no correlation with infection, hepatic disorder, or malnutrition. Most cases manifested chronic pulmonary disease, usually emphysema. Twelve patients had

malignant neoplasms; in 2 there were small carcinomas of the prostate, and in 2 there were hepatomas. Other pathologic observations did not differ significantly from those which might be encountered in a similar group of patients without amyloid.

Clinically, there was no correlation with hypertension, arteriosclerosis, anemia, urinary tract abnormality, azotemia, or malnutrition. Of 11 patients in whom serum protein determinations were recorded, in only 2 was there elevation of the serum globulin. Most individuals in the second test period had received antibiotic therapy, but a few apparently had not. About half of the patients were given digitalis preparations, but only a few received mercurial diuretics. Electrocardiographic features were nonspecific in 27 cases, generally indicating nonspecific myocardial damage. Only one patient revealed low voltage.

It is concluded that senile cardiac amyloidosis represents a unique pathologic entity which should be separated from the secondary and generalized primary forms. Significant features include a limitation of amyloid deposit almost entirely to the myocardium and its occurrence almost exclusively in aged individuals. A careful survey of possible contributing factors reveals no plausible cause. Inexplicably, there appears to have occurred in recent years an increase in incidence limited entirely to individuals in the ninth and tenth decades.

A Method for Producing a Thrombus *In Vitro*. A. B. Chandler, Medical College of Georgia, Augusta, Ga.

A thrombus forms in a moving stream of blood by selective deposition, at a fixed point on the endothelium of a vessel, of platelets, granulocytes, and fibrin. A blood clot forms in a stagnant column of blood or in a test tube, and its development is not comparable to that of a thrombus in a moving stream of blood. A simple experimental technique based on the principle that a thrombus forms in a moving stream of blood has been devised for the production of thrombi *in vitro*. All experiments were carried out utilizing the blood of healthy adult human males.

An *in vitro* thrombus was produced by causing a column of blood to flow in a circular polyvinyl tube. One ml. of venous blood was drawn directly into a tube 25.0 cm. in length and 0.375 cm. in diameter. The 2 ends of the tube were joined by an outside collar, and the circular tube was rotated on a slanted turntable at 17 r.p.m. The thrombus formed in the moving blood column as it slid along the walls of the rotating tube. When the thrombus formed (10 to 23 minutes), the lumen became occluded and the blood column moved in the direction of rotation of the tube; this established an end point. The thrombus was composed of interlocking clumps and bands of agglutinated platelets connected by strands of fibrin. The spaces between the platelet clumps contained granulocytes and a few red cells. The thrombus formed *in vitro* by this method was strikingly similar in structure to that of a thrombus arising *in vivo*.

The possible applications of this method to the study of thrombosis will be presented.

The Histologic Diagnosis of Amniotic Fluid Embolism. Harold D. Attwood, Yale University School of Medicine, New Haven, Conn.

The histologic diagnosis of amniotic fluid embolism can be made easily and with certainty by the use of a method combining Alcian green staining of mucin with phloxin staining of squames. The method is simple and of high specificity. The tissues from 16 patients with amniotic fluid embolism were examined, using this staining technique. A considerable degree of contamination of the maternal pulmonary vessels with fetal debris was demonstrated in the rapidly fatal cases.

This was much greater than would have been suspected in hematoxylin and eosin stained preparations.

THE PATHOLOGY OF THE LOWER ESOPHAGEAL RING. H. Edward MacMahon* and Richard Schatzki, Tufts Medical School, Boston, Mass., and Mount Auburn Hospital, Cambridge, Mass.

The purpose of this brief report is to describe for the first time the necropsy findings in a patient with a symptomatic "lower esophageal ring." A roentgenologic diagnosis had been established and repeatedly confirmed over a period of 9 years. The lesion appeared in the roentgenogram as a narrow, deep, circular indentation in the lumen of a distended lower esophagus. It lay at the junction of the esophageal and gastric mucosa and appeared as a nearly symmetrical, circular, wedge-shaped shelf or diaphragm that protruded from the wall into the lumen in a plane that was almost vertical to the long axis of the esophagus. Histologically it was found to represent little more than a wide mucosal fold covered above by stratified squamous esophageal mucosa and below by gastric mucosa. A delicate annular ring of smooth muscle lay immediately beneath the epithelium covering the innermost free margin. The anatomic construction of this diaphragm, known as "a lower esophageal ring," offers a reasonable basis for its correction by therapeutic rupture.

THE PRODUCTION OF VALVULAR LESIONS BY MECHANISMS WHICH ELIMINATE SENSITIVITY FACTOR. Alfred Angrist,* Masamichi Oka, Komei Nakao, and Jeanne Marquiss, Albert Einstein College of Medicine, New York, N.Y.

Intravalvular alterations and superimposed vegetations have been produced by nonspecific forms of stress. These were accomplished by exposing rats and rabbits to high altitude, tumbling, cold room, and wet bedding; high altitude and cold room, parabiosis, and several steroid hormones. Some of the animals had had adrenalectomy; others were intact. The lesions included diffuse edematous valvular swelling, localized edema and thickening, the appearance of platelet thrombi, and inflammatory changes characterized by increased cellularity, prominence of Anitschkow elements and palisading of reactive cells. Histochemical procedures and enzyme studies for phosphatase and esterase showed no alterations.

Stress in an altered endocrine state (adrenalectomy) seems to cause variation in the form of the vegetation induced; platelet thrombi predominated in some instances, and alterations of the valve itself, particularly with the occurrence of an inflammatory reaction, in others.

THE VALUE OF MUCOPOLYSACCHARIDE HYDRATION IN THE DEMONSTRATION OF MICROBIAL CAPSULES IN PARAFFIN SECTIONS: WITH SPECIAL REFERENCE TO *Cryptococcus neoformans* AND PNEUMOCOCCI.† Robert W. Mowry,* University of Alabama Medical Center, Birmingham, Ala.

The morphologic recognition of *Cryptococcus neoformans* requires demonstration of its mucinous capsule which, like that of the pneumococcus, is rich in acidic polysaccharide. At an earlier meeting (1956) we reported that capsules of *C. neoformans* were consistently stainable with Alcian blue and were metachromatic with toluidine blue in paraffin sections. Both methods stain acidic polysaccharides. Capsules of pneumococci in sections were also frequently stainable. Dried films of purified capsular polysaccharide from these organisms stained similarly. However, the capsules of cryptococci seen in stained paraffin

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sections were thin and corrugated in outline, compared to their plump appearance when they were stained with India ink. These defects in capsule demonstration have proved to be largely preventable. Smears of *C. neoformans* examined after Alcian blue staining while wet showed capsules that were thicker and smoother than those observed in paraffin sections. But the capsules became shrunken and rough in outline after dehydration and mounting in resin. The capsules became expanded again when exposed to water or were "hydrated." Mounting in glycerin jelly preserved the desired capsular appearance.

Paraffin sections from 10 different cases of cryptococcosis were stained by various methods and mounted in glycerin jelly for comparison with duplicate sections mounted in resin after dehydration. Sections mounted in glycerin jelly showed capsules that were much thicker and more clearly defined in each instance. Capsules of pneumococci were also studied. Alcian blue and the colloidal iron stain, unlike toluidine blue, did not fade in glycerin jelly. It is quite possible that the morphologic features of mucin-rich tissue structures other than microbial capsules may be rendered more lifelike by a deliberate effort to maintain some degree of hydration instead of imposing drastic dehydration after staining of paraffin sections.

THE APPLICATION OF TETRAHYDROFURAN (THF) TO NECROPSY AND SURGICAL PATHOLOGY TISSUE. M. Daria Haust, Robert H. More* and G. F. Kipkie,* Queen's University and Kingston General Hospital, Kingston, Ont., Canada.

A year ago several aspects of the experimental use of Tetrahydrofuran (THF) in research work were reported. During the past year a successful attempt was made to introduce this dehydrating and clearing medium for routine use in both the necropsy and surgical pathology services. The method can be summarized as follows: (1) On the necropsy service the automatic tissue processor operated overnight. During this time not only were the tissues adequately dehydrated and cleared by THF, but additional fixation was obtained by adding 2 containers with fixative (2 hours each) to fill the unused time between 5 P.M. and 9 A.M. (2) In surgical pathology where adequate fixation of the fresh material received from the operating room is imperative in order to achieve good sections, the addition of the above-mentioned containers with fixative was beneficial. (3) If frozen sections are not adequate and rapid paraffin sections are required, a short schedule has proved to be useful. It required only 4 to 4½ hours time for fixation, dehydration, clearing, and infiltration. Although the quality of the permanent sections processed in this fashion was approximately equal to that obtained with those processed on the longer THF schedule, caution should be exercised in extending this short schedule to routine use until more is learned about preservation of blocks after so short a period of treatment. (4) THF also successfully replaced the various dehydrating media used in the study of exfoliative cytology and the dehydrating and clearing media for paraffin sections before staining.

The high quality of tissue preservation and staining after the use of THF will be illustrated by color photomicrographs.

A HISTOLOGIC STUDY OF THE UPPER SMALL INTESTINE OF MICE IMMUNIZED WITH PRE-ADULT IRRADIATED *Trichinella spiralis* LARVAE. John E. Larsh, Jr., and George J. Race,* School of Public Health, University of North Carolina, Chapel Hill, N. C., and University of Texas Southwestern Medical School, Dallas, Texas.

Evidence indicates that immunity to *Trichinella spiralis* during the intestinal phase of parasitization results from the action of specific antibodies against the parasite and is associated with a localized cellular inflammatory reaction which develops at the point of parasitic invasion. In adult immunized mice, a severe

inflammatory reaction developed in the upper small intestine at the site of parasitic localization. Thereafter, the worms were rapidly eliminated from the intestine. In immunized animals, the peak of the reaction occurred at 4 days, while in nonimmunized mice the peak was reached at the eighth day. A low titer of antiparasitic antibody was demonstrable before the elimination of the worms from the body. However, in nonimmunized immature mice, the inflammatory process developed slowly, suggesting that very young mice acquired immunity to *T. spiralis* less readily than the older mice. In the current immunization experiment, using trichinae larvae irradiated with 7,000 r. to prevent maturation of the larvae beyond the pre-adult phase (not over 48 hours), the nonimmunized controls yielded significantly greater numbers of adults (av. 379.5) at 7 days than could be recovered from mice immunized with normal larvae (av. 262.3, p–0.001); or from mice immunized with irradiated larvae (av. 314.0, p–0.005). The characteristic intestinal inflammation was slower in its development and was less marked in mice immunized with irradiated larvae, suggesting that the development of immunity to the parasite was less when the mouse was immunized with pre-adult irradiation-stunted larvae.

The serologic titer (hemagglutination test) in the control group was negative, with irradiated larvae it was 1:640, and in the normal larvae group, 1:2560.

OCCULT TUBERCULOUS ENDOBRONCHITIS IN SURGICALLY RESECTED LUNG SPECIMENS. J. Robert Thompson and Geoffrey Kent, Municipal Tuberculosis Sanitarium, Chicago, Ill.

A study of the histologic lesions in the surgically resected pulmonary tissue procured from 344 consecutive patients with tuberculosis is presented. Preoperative endoscopic examination failed to reveal the presence of any bronchial lesions. Blocks of tissues were taken to include the bronchus draining the principal parenchymal lesion at 3 different levels: (a) the juncture of the bronchus with the parenchymal lesion, (b) the stump or point of amputation of the bronchus or branch bronchi, and (c) a point midway between the stump and the tuberculous lesion. Prior to fixation of the specimens, material was aspirated from the bronchial tree for bacteriologic study. Histologic evidence of tuberculous endobronchitis was found in 12.8 per cent of the cases and of this group 14.4 per cent showed evidence of active tuberculosis in the peribronchial lymph nodes as compared to only 2 per cent lymph node tuberculosis in cases without tuberculous endobronchitis. Positive cultures were obtained in 64 per cent of the specimens with demonstrable endobronchitis as compared to only 6.5 per cent of the group without endobronchitis. Three pathogenetic factors are set forth, and the pathologic features of tuberculous endobronchitis described.

THE VARIATION IN VIRULENCE FOR GOLDEN HAMSTERS OF DIFFERENT STRAINS OF MYCOBACTERIA.† Ann Pollak,* Albert Einstein College of Medicine, New York, N. Y.

It has been shown previously that hamsters are uniformly susceptible to infection with at least 2 different strains of Mycobacteria, viz., the tubercle bacillus and an atypical acid-fast organism previously called the "Yellow Bacillus" and now known as *Mycobacterium kansasii*. The latter organism also produces serious and occasionally fatal disease in human beings. In this experiment these observations were extended by comparing the virulence for golden hamsters of several different strains of Mycobacteria: Two strains of *Mycobacterium kan-*

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sasii, the $H_{37}Rv$ strain of the human tubercle bacillus, the Ravenel strain of the bovine tubercle bacillus, the Rosenthal strain of bacillus Calmette-Guérin, the Sheard strain of the avian tubercle bacillus, the vole bacillus and finally *Nocardia asteroides*. Twenty-four animals were placed in each experimental group. Each group was inoculated with one of the various test organisms.

The results showed that a whole spectrum of pathogenicity was produced by the different strains of organisms tested. Thus all of the animals injected with the bovine tubercle bacillus were dead within 2 months. All but 2 of the animals given the human tubercle bacillus or *Mycobacterium kansasii* died within 2 to 4 months after inoculation. On the other hand, by the end of the sixth month, between 75 and 100 per cent of the other animal groups were surviving. The lesions produced were similar in all cases, but larger and more numerous in groups injected with more virulent organisms. Although all of the animals received inoculating injections intraperitoneally, the human and bovine tubercle bacilli were more apt to produce extensive lesions in the lungs than were the other organisms.

DIHYDROCHOLESTEROL-INDUCED LAPIN CIRRHOSIS. William H. Hartmann, Joseph C. Sieracki, William T. Beher and Robert C. Horn, Jr.,* Henry Ford Hospital, Detroit, Mich.

Stimulated by reports indicating the reduction of hypercholesterolemia following the administration of dihydrocholesterol (a naturally occurring sterol), 60 rabbits were maintained on a diet which included this compound. Some animals died and others were sacrificed at varying intervals of time up to one year. Biliary tract calculi were observed in all rabbits within 10 weeks. This was followed 3 to 4 weeks later by the development of an obstructive type of cirrhosis which progressed to almost total destruction of hepatic tissue and, in some animals, caused death. The severity of the changes appeared to be roughly proportional to time. Extensive alterations were noted in other viscera. Various chemical determinations were performed throughout the course of the experiment.

UTERINE ERYTHROCYTOSIS. Alexander Niedzewiedz, Abraham M. Frumin, and David R. Meranze,* the Albert Einstein Medical Center, Southern Division, Philadelphia, Pa.

A 59-year-old white female, gravida I, Para I, had a large pelvic mass for 5 years, and radiologic changes in both lungs, consistent with secondary polycythemia. The peripheral blood revealed erythrocytosis. Both the erthrocytosis and lung lesions reverted to normal on removal of a large myomatous uterus.

This case, and the 4 similar cases in the literature, were reviewed, and the histologic findings were compared with those of 100 consecutive uterine myomas removed at the Southern Division of the Albert Einstein Medical Center. Sections from the myomas of the patients exhibiting secondary polycythemia revealed increased vascularity, but were by no means distinguishable from sections of the large myomas from patients without this condition. Oophorectomy was not related to the subsidence of the erythrocytosis since, in one of the cases published, total hysterectomy alone was done. In addition to the routine blood studies, a pre- and post-operative blood volume determination was carried out, utilizing radioactive isotopes. A tremendous reduction in cell volume was observed following hysterectomy.

Oxygen saturation values of the arterial blood, and oxygen saturation of the venous blood leaving the uterus before and after operation, are suggested as procedures in other cases in order to further elucidate the pathogenesis of the

phenomenon. Experimental investigation of uterine venous blood and the uterine tissue in laboratory animals, would also be helpful in this respect.

THE MECHANISMS OF FORMATION OF VARIOUS HISTOLOGIC PATTERNS AS OBSERVED IN MAMMARY TUMORS OF MICE. Hemprova Ghosh, Washington University School of Medicine, St. Louis, Mo.

The material for demonstration will be limited to the microscopic study of spontaneous and transplanted mammary carcinoma in the C_3H strain of mice. The process of formation of various tumor structures including papillary and papillary cystic patterns, those resembling hemangiopericytoma and others are described. Cellular lysis, degeneration and necrosis in the tumors were found to play as important a role as cellular multiplication in the formation and interplay of different tumor patterns. A significant consequence of these activities was to provide space for regenerating tumor cells. The potentialities of the neoplastic cells to differentiate into cells and tissues indistinguishable morphologically from the normal ones were also demonstrated. The observation of the formation of endothelium-like membranes by the metastatic tumor cells while growing inside the cardiac cavity was of special interest, as this virtually reproduced the appearance of tumor tissue lying in transversely sectioned lymphatic channels.

MALIGNANT MESENCHYMOMA (OF THE HEMANGIOBLASTOMYXOMATOUS VARIETY) IN A FIVE-YEAR-OLD BOY. G. F. Kipkie* and M. Daria Haust. Queen's University and Kingston General Hospital, Kingston, Ont., Canada.

By definition, a malignant mesenchymoma is a neoplasm consisting of 2 or more unrelated mesenchymal components which are malignant. This group thus comprises a multitude of tumors of different composition, some of which are encountered quite frequently, others rarely. To the latter group belongs the hemangioblastomyxoma. It is the purpose of this presentation not only to report on this rather rare tumor, but also to add evidence to the dysontogenetic theory of its origin and by means of careful morphologic study to reproduce its pathogenesis.

The case was that of a 5-year-old boy, the last child of elderly parents. Five months prior to his death a mass was noted at the left mandibular angle. This grew very rapidly, and at the time of biopsy (6 weeks later) was found to have completely destroyed the left mandibular ramus. Microscopic examination revealed predominantly a loose and myxomatous matrix in which were embedded numerous vascular channels varying in size and content. Some of the channels contained blood, others contained eosinophilic, protein-rich material. Characteristically, there was absence of a well developed wall in the vascular channels. Surrounding them were cells which appeared to decrease in number in the loose matrix with greater distance from the normal appearing structures. Among them were present all forms of transformations from the typical mesenchymal angioblast to the endothelial angioblast. As in the observations of F. G. Sabin (1920), the lumen of a vessel was formed either by liquefaction of the cytoplasm of a single cell or by liquefaction at the center of the angioblastic mass. Special stains revealed that these small vascular spaces were outlined by reticulum fibers and contained either red blood cells or hemolyzed blood. An attempt is made to suggest a dysontogenetic origin of the tissue.

LYMPHOMA OF PLASMA CELL LINEAGE (EXCLUSIVE OF MULTIPLE MYELOMA). Dante Campagna-Pinto and Russell P. Sherwin, Mallory Institute of Pathology and Massachusetts Memorial Hospitals, Boston, Mass.

A morphologic study of malignant lymphoma was carried out, using necropsied cases from the Boston City Hospital, New England Deaconess Hospital, and Massachusetts Memorial Hospitals. A 20-year period (1937 to 1957)

was covered. From this review it was apparent that young and mature plasma cells were often significant components of certain forms of lymphoma. The following studies were made on this group: (a) clinico-pathologic correlation; (b) hematologic and clinical laboratory studies (whenever available); (c) histochemical investigations; and (d) chemical studies of formalin-fixed tumor homogenates. The results of the study indicate that lymphoma of plasma cell lineage forms a distinct and, except for the classical, mature plasmocytomas, a hitherto poorly recognized group. Mixed forms were also found in which cells which were not plasma cells predominated. The finding of plasmocytoid cells in some lymphocytic lymphomas was confirmed. The latter group has already been reported as showing serum and urine protein abnormalities of the type usually associated with plasma cell proliferation.

THE PATHOGENESIS OF EXPERIMENTAL LYMPHOBLASTOMA INDUCED IN C_3HEB MICE BY MEANS OF CELL-FREE BRAIN FILTRATES. Paul B. Szanto,* Steven O. Schwartz, Harold M. Schoolman and Wilma Spurrier, The Hektoen Institute for Medical Research of Cook County Hospital, Chicago, Ill.

Either intracerebral or intraperitoneal inoculations of cell-free filtrates prepared from a pool of brains of 3 leukemic C_3Heb mice were introduced into young adult C_3Heb mice. For controls, cell-free filtrates prepared from the brains of 3 nonleukemic C_3Heb mice were injected into comparable animals. In addition, a group of mice received a tumor cell suspension intraperitoneally. Each day at least 2 animals from each group were sacrificed. The locations of the various lymph nodes were labeled, and all organs were preserved in various fixatives. Sections were stained with hematoxylin and eosin, the Masson trichrome stain, and cresylecht violet; others were impregnated with silver. The sections were also studied by phase and fluorescent microscopy. Touch preparations and blood and marrow smears were stained with Wright's stain and counterstained with Giemsa stain. Some variation of the degree and distribution of the lymphoblastoma was present in the obviously affected animals sacrificed between the seventh and eleventh days. The main purpose of the present study was to evaluate the earliest changes which were found during the fourth and fifth days after inoculation. In these animals, the developing lymphoblastoma could be demonstrated simultaneously in various distant organs, thus indicating multicentric origin. The architecture of the lymphatic tissue was preserved despite the presence of tumor cells. The expected changes unfolded progressively and simultaneously in various organs. The other series of experiments were carried out, using Swiss and AKR mice; in these, similar results were demonstrated.

THE PATHOGENESIS OF EXPERIMENTAL BRONCHIOLITIS OBLITERANS. Thomas J. Moran* and H. Richard Hellstrom, Presbyterian Hospital and Veterans Administration Hospital, Pittsburgh, Pa.

This study was designed to find a simple method of producing focal bronchiolitis obliterans, to study the pathogenesis of this condition, and to determine whether or not cortisone might prevent or modify its development. The intratracheal injection of 1.0 to 1.2 per cent nitric acid in rabbits caused death from acute pulmonary edema or chemical pneumonia at varying times in approximately two thirds of 113 rabbits. The animals were divided into 3 groups: one group was treated with intramuscular injections of penicillin and streptomycin; one group received intramuscular injections of cortisone, penicillin and streptomycin; and one group received no treatment. The mortality rates and duration before death from acute pulmonary edema or from chemical pneumonia in the 3 groups were identical. In surviving animals, however, 13 of 26 animals which had not received cortisone developed focal bronchiolitis obliterans, while only

one of the 12 cortisone-treated animals developed these lesions. The pathogenesis of the bronchiolitis obliterans produced by the endobronchial instillation of nitric acid was traced. The observations support the viewpoint that the lesion occurred as a direct result of the healing by fibrosis of the initially damaged bronchiolar mucosa rather than from long-continued inflammation in the bronchiolar wall.

CHANGES IN THE BRONCHIAL EPITHELIUM OF MICE AFTER EXPOSURE TO CIGARETTE SMOKE. (A CORRELATED HISTOLOGIC, CYTOLOGIC AND CYTOCHEMICAL STUDY).† Cecile Leuchtenberger, Rudolf Leuchtenberger, William Zebrun and Patricia Shaffer, Western Reserve University, Cleveland, Ohio.

This report deals from a histologic, cytologic and cytochemical point of view with the sequence of events which take place in the bronchial epithelium of mice after exposure to cigarette smoke in a smoking chamber. When mice were exposed to cigarette smoke from approximately 4 to 6 cigarettes daily for a period of 2 months or longer, a bronchitis which was associated with a proliferation of the bronchial epithelium developed. There appeared to be a stepwise sequence of histologic and cytologic changes ranging from normal resting epithelium to swelling, proliferation, and finally, to atypical hyperplasia of the epithelium ("carcinoma *in situ*"). Cytochemical changes consisted of a similar stepwise sequence of events in the bronchial epithelium and lungs and furthermore indicated that the intracellular chemical changes occurred before structural alterations manifested themselves in cells and tissues. By the use of microspectrophotometry and interference microscopy which permitted the chemical analysis of single cells *in situ*, it was possible to compare and correlate directly the microscopic appearance and chemical composition of each cell analyzed. After exposure to cigarette smoke, bronchial epithelial cells with normal microscopic appearance and lung parenchyma contained an increased quantity of protein and dry weight. This was followed later by a gradual doubling of the deoxyribose nucleic acid (DNA).

The discussion will deal with the question whether the cytochemical derangement may be considered an early stage in the sequence of events preceding the atypical morphologic alterations of cells and tissues, and with the problem of reversibility of the atypical changes.

READ BY TITLE

ON THE IMPORTANCE OF THE ATYPICAL ENDOMETRIAL CHANGES ASSOCIATED WITH THE PRESENCE OF CHORIONIC TISSUE IN THE DIAGNOSIS OF PREGNANCY. Javier Arias-Stella,* Faculty of Medicine, Lima, Peru.

The atypical endometrial changes accompanying chorionic tissue constitute significant histologic clues to the diagnosis of pregnancy. In 44 patients with ectopic pregnancy from whom the endometrium was available for study, atypical epithelium was found in 22 cases. In 11 instances, pregnancy was suggested, before laparotomy, on the basis of the atypical change in the endometrial biopsy tissue. In some of these, ectopic pregnancy had not been suspected clinically. Among the 22 specimens with positive findings, 11 showed the marked atypia I have described in previous papers. This was characterized by focal abnormalities of the endometrium with a pattern defined as "minimum atypia." All the features of the lesion were present but in minor degree. Correlative studies demonstrated that the atypical alteration occurred only when chorionic villi were not totally involuted. Repeated biopsy specimens from single patients

† This study was supported by a grant from the Tobacco Industry Research Committee.

showed that the so-called "mininum atypia" could proceed to a "regression phase" with more definite alterations. This might represent a response to a weaker stimulus. After involution of chorionic tissue, the atypism in the surface epithelium remained for a longer period of time than that in the glands. The question should be raised as to how slight the endometrial atypism may be and still maintain its diagnostic significance. It was found that the abnormal endometrium occurred in instances of normal uterine pregnancy. Based on the presence of "minimum atypia" the presumptive diagnosis of pregnancy has been made in 3 patients later proved to have uterine pregnancies. In none of these was chorionic tissue present in the biopsy specimen. In two instances biopsy was made because of suspicion of uterine tumor. The third case was a patient under investigation for sterility.

BOVINE OCULAR SQUAMOUS CARCINOMA ("CANCER EYE") AND ITS BENIGN PRECURSOR LESIONS IN THE LIGHT OF RECENT TISSUE CULTURE AND ELECTRON MICROSCOPE STUDIES. Leon Dmochowski, John A. Sykes, E. Staten Wynne and William O. Russell,* The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

The pathologic anatomy of bovine ocular squamous carcinoma ("cancer eye") and its benign precursor lesions has been described by Russell, Wynne and Loquvam (1956). The findings have been extended by studies on 1,200 living animals observed for periods up to 4½ years. In the conjunctival sac, carcinomas develop from hyperplastic plaques, some passing through a papillomatous stage. The plaque-papilloma-carcinoma sequence, as well as the high incidence of multiple lesions and bilateral involvement, suggested the desirability of correlating studies of anatomic pathology, electron microscopy, and tissue cultures to evaluate the possibility of a transmissible factor. Investigations with the electron microscope showed that the spherical, eosinophilic, intranuclear bodies observed by Russell and co-workers in all 3 types of conjunctival lesions were nucleoli of characteristic shape and size. Representative electron micrographs of the lesions will be shown.

Difficulties associated with obtaining primary cellular outgrowth of these lesions *in vitro* will be discussed. From 70 lesions, primary growth was obtained in 40. Cultures of a number of lesions were maintained for 2 to 7 months and for 2 to 23 passages. Cells derived from 14 different lesions are at present in culture: 11 came from plaques, 2 from papillomas, and 1 from a carcinoma. Cellular changes observed in some of these cultures, in both stained and living states suggested the presence of a cytopathogenic agent in the lesions from which the cultures were derived. The significance of these observations will be discussed.

FURTHER OBSERVATIONS ON THE EFFECTS OF DIETARY SODIUM DEFICIENCY ON RENAL JUXTAGLOMERULAR CELLS AND ADRENAL CORTEX (RAT, DOG, CAT).† P. M. Hartroft, L. Newmark and J. A. Pitcock, Washington University School of Medicine, St. Louis, Mo.

As previously reported, the degree of granulation of juxtaglomerular cells was increased in rats fed a sodium-deficient diet. More recently, by using younger rats and more severe sodium restriction, it was possible to produce hyperplasia of juxtaglomerular cells in addition to an increase in granulation. In these rats, plasma sodium fell and the zona glomerulosa of the adrenal cortex increased to 8 times its normal width.

These observations have been extended to dogs and cats. In reports based on

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studies in mature dogs, peritoneal dialysis was necessary to lower the serum sodium. In the present investigation, sodium depletion was produced by dietary means alone in young growing puppies. Plasma sodium fell during the first 3 to 4 weeks to levels of approximately 125 mEq per l. and thereafter remained low as growth continued. Litter mate beagle puppies were used for these experiments in order to provide adequate histologic controls and assure absence of spontaneous renal lesions. Sections of kidney and adrenal were obtained by biopsy (3 weeks to 2 months) and at necropsy when the dogs were sacrificed (up to 9 months). Without exception, the zona glomerulosa became hyperplastic and increased in width; this was accompanied by hyperplasia and hypergranulation of renal juxtaglomerular cells. Similar results were obtained in preliminary studies on kittens. Of the 3 species studied, the dog was found to respond to sodium deficiency with the most striking alterations. The histologic features will be discussed in relation to electrolyte balance and adrenal steroid secretion.

INTRACELLULAR ("PSEUDO-ALCOHOLIC") HYALIN IN EXPERIMENTAL DIETARY CIRRHOSIS OF RATS AND MICE.† W. Stanley Hartroft,* Washington University School of Medicine, St. Louis, Mo.

The late F. B. Mallory discovered that coarse hyalin granules, clumps, or skeins of strongly acidophilic material were frequently present within the cytoplasm of hepatic cells of fatty cirrhotic livers in patients with alcoholism. Although cirrhosis of choline-deficient animals resembles in many respects that associated with alcoholism in man, intracellular hyalin has not been emphasized in the animal. Smudges and ill-defined globules of phloxine-staining, intracytoplasmic material have been observed in small amounts in the livers of choline-deficient rats. In mice fed a hypolipotropic diet (without alcohol) for 7 months or more, abundant amounts of intracellular hyalin were present. Skein forms were not frequently seen in the mice, but other forms described in man were numerous. As in the man with alcoholism, necrotic liver cells and small collections of neutrophils were encountered in the cirrhotic livers of mice but rarely in those of rats. It has been suggested that cells containing hyalin may undergo necrosis and thus provoke this reaction in the livers of patients with alcoholism. The correlation between the amounts of hyalin and the numbers of necrotic foci in the livers of rats and mice supported this hypothesis. Treatment of mice with cirrhosis for several months with dietary choline resulted in complete disappearance of intracellular hyalin although ceroid persisted.

MESONEPHRIC CARCINOMA OF THE OVARY. Saul Kay* and Randolph H. Hoge, Medical College of Virginia, Richmond, Va.

This paper serves to confirm the concept of the mesonephric origin of the Schiller ovarian tumor. Nine cases are presented which represent an incidence among ovarian tumors of 13.6 per cent during a 7-year period of study. The tumors were characterized by tubular structures which often resembled Wolffian duct tubules; in some, dichotomous branching was a prominent feature. The tumor cells frequently had clear cytoplasm which in most instances stained positively for mucin. Glycogen could also be detected when sought for. An exaggeration of the cytoplasmic vacuolization resulted in a microscopic pattern of hypernephroid carcinoma which resembled the clear cell carcinomas commonly found in the kidney. When vacuolization was extreme, lipid material appeared to be the principal component in the cells. These neoplasms are malignant and presage a grave prognosis should capsular invasion and adherence to adjacent structures be present.

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ELECTRON MICROPSCOPY OF THE GROWTH CYCLE OF HUMAN STRAIN OF SALIVARY GLAND VIRUS GROWN IN TISSUE CULTURE.† Sarah A. Luse* and Margaret G. Smith,* Washington University School of Medicine, Saint Louis, Mo.

The purpose of these experiments was to study the growth cycle of a human strain of salivary gland virus and the concomitant cytologic alterations occurring during development of intracellular viral forms. Fibroblasts derived from human myometrium were inoculated with virus. Control and infected cultures were prepared for electron microscopy by the usual methods at progressive intervals following inoculation. Control fibroblasts are elongated cells with 1 to 2 nucleoli, scant mitochondria and a variable amount of ergastoplasm. Within 15 to 30 minutes after inoculation with virus, ergastoplasmic sacs became dilated and filled with finely granular, pale, homogeneous material. By 2 to 4 hours Golgi membranes were increased, ergastoplasmic sacs were still dilated, and cellular contours were distinctly rounded, rather than elongated as in control cultures. Initial nuclear alteration at 24 hours consisted of multiple collections of clumped nuclear chromatin surrounded by pale halos. The first particles, considered to be viral particles, were seen in the nucleus at 3 days. Nuclear particles had increased in number by 5 days and formed a distinct nuclear inclusion surrounded by a pale halo. Not until 5 days were cytoplasmic particles encountered that could be considered to be of viral nature. Later stages of infection (10 to 30 days) showed the presence of increasing numbers of viral particles in both cytoplasm and nucleus with degenerative changes in mitochondria. Increasing numbers of extracellular viral particles appeared after 5 days following the dissolution of degenerated cells.

CHANGES IN SERUM GLOBULINS DURING INFLAMMATORY PROCESSES. Crichton McNeil,* Holy Cross Hospital, Salt Lake City, Utah.

It is now well known that deficiencies of gamma globulin of both hereditary and acquired nature are frequently accompanied by poor natural resistance to infection. Treatment with gamma globulin seems to hold the infectious disease process in abeyance until natural immunity factors can recover. We have now examined over 4,000 electrophoretic tracings in all types of disease entities and have recorded many follow-up tracings during recovery. The material showed that in addition to gamma globulin loss, there was frequently an elevation of alpha, and particularly alpha$_2$ globulin fractions which reflected the severity of the inflammation. When the patient recovered, either spontaneously or as the result of specific therapy, the alpha fractions returned to normal levels. Typical case studies in both children and adults will be used to illustrate the contention that stress plays an important role in the protein pattern.

THE HYALINE MEMBRANE SYNDROME OR DISEASE: A DANGEROUS MISNOMER. J. Tannenberg,* Genessee Laboratory, Batavia, N.Y.

The designation "hyaline membrane syndrome" is considered a dangerous misnomer because of the hopelessness that is implied when the deposit of hyaline membranes in the lung is regarded as the main cause for a clinical state. The use of the term diverts attention from the real causes of the disorder. According to earlier experimental investigations published by J. Tannenberg and Max Pinner, uncomplicated pulmonary atelectasis can only be produced by compression of the lungs or obstruction of the upper airways. Atelectasis complicated by exudate, however, can be produced by infection and also by narrowing or obstruction of pulmonary arteries or veins. The very presence of hyaline membranes filling

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alveoli and bronchial ducts is incompatible with simple atelectasis and places the condition into the category of atelectasis complicated by circulatory disturbances.

Material of the same appearance histochemically as the hyaline membranes in the lungs was found to cover single or groups of placental villi in more than 25 mature placentas procured from consecutive Cesarean sections. This indicated that these membranes may be formed in exudate derived from blood.

Damage to the circulatory system sufficient to produce pulmonary exudate in newborn infants may be expected in one or all of the following 3 circumstances: (1) Injury to the central nervous system respiratory and circulatory centers (birth trauma with hemorrhage followed by spreading edema, or functional damage resulting from labile and low level of blood sugar in the first few days of life, particularly in premature infants and infants of diabetic mothers). (2) Injury to the heart (low blood sugar level and increased red cell concentration in the blood). Recent experiments in our laboratory showed that the viscosity curve of the blood increased unduly when the hematocrit values markedly exceeded the normal values of the species. This was particularly the case in premature newborn infants, and placed extraordinary demands on the heart. (3) Impaired peripheral pulmonary circulation (weakened cardiac and respiratory action and damage possibly due to therapeutically applied high oxygen concentration). In experimental observations on the lung of the frog, it was reported that oxygen-saturated Ringer's solution produced definite and measurable contraction of the small arterioles in the lung.

In diverting attention from the hyaline membranes to the circulatory system in the syndrome in question, a more hopeful way of combating it is opened. Routine microdeterminations of the blood sugar in newborn infants and determinations of the micro-hematocrit in the first few days of life, particularly in premature infants, infants delivered by Cesarean section, and infants of diabetic mothers, may indicate an effective treatment for this, thus far, hopeless condition.